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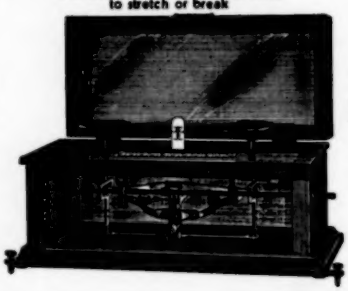

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A.PH.A. CONVENTION INFORMATION

Very shortly the members of the AMERICAN PHARMACEUTICAL ASSOCIATION will receive by mail the preliminary announcement of the program for the Boston Convention. This announcement will include an application for hotel accommodations similar to that which is printed on the third cover page of THIS ISSUE of the *Scientific Edition* of the JOURNAL.

It is expected that sufficient accommodations will be available at the Statler Hotel in Boston, which is the Headquarters Hotel for the Convention, for all who wish to be assigned there. All hotel accommodations must be arranged for through the Housing Bureau which has been set up for that purpose, and it will be most helpful if members of the ASSOCIATION will use the official blank in making their requests for reservations.

The program will follow the customary arrangement, with three General Sessions, to be held on Tuesday evening, Thursday morning, and Friday evening, respectively, of the convention week.

At the First General Session to be held on Tuesday evening August 24, President F. Royce Franzoni will give his presidential address, which will be followed by a reception and dance in honor of President and Mrs. Franzoni.

The Second General Session will be held on Thursday morning, August 26, and will include several addresses on topics of current importance by outstanding speakers. This session will also be marked by the award of the Ebert Prize, the Kilmer Prize, the Iodine Educational Bureau, Inc. Award, and the address of the President-Elect, Mr. Newell Stewart.

Four sessions of the House of Delegates will be held on Tuesday afternoon, August 24, Wednesday morning August 25, Friday morning, and Friday evening, August 27, respectively.

The meetings of the House of Delegates on Tuesday and Friday evening will immediately precede the General Sessions of the ASSOCIATION.

Members of the House of Delegates will be requested to supply nominations on official nominating forms for the offices of President, First Vice-President, Second Vice-President, and Chairman and Vice-Chairman of the House of Delegates. These forms will be sent to each member by mail and must be returned by June 22.

The members of the House of Delegates are also expected to send proposed resolutions to the office of the ASSOCIATION on forms provided for that purpose. It is important that the committee on resolutions shall have sufficient time to review resolutions submitted, in advance of the meeting, so that they may be acted upon as early in the sessions of the House of Delegates as possible.

The five Sections of the ASSOCIATION will hold meetings for the reading of papers on Wednesday, Thursday, and Friday, August 25, 26 and 27. Those who wish to submit papers to be read at these Section meetings, should contact the respective section secretaries whose names and addresses are listed on Page VI.

As has been customary in previous years, the American Association of Colleges of Pharmacy, the National Association of Boards of Pharmacy, the American Society of Hospital Pharmacists, The American College of Apothecaries, and The National Conference of State Pharmaceutical Association Secretaries, will hold meetings on Sunday, Monday and Tuesday, August 22, 23 and 24 of the Convention Week.

Entertainment features will include the Annual Banquet of the ASSOCIATION on Thursday evening, August 26, and the customary entertainment features for the ladies throughout the Convention week.

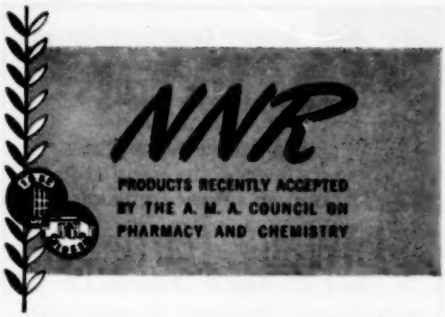
Biennial Membership List

The biennial *Supplement* to the *Scientific Edition* of the JOURNAL, carrying the full list of active members in good standing will be published with the June issue of THIS JOURNAL.

There is still time for members in arrears in their dues to be listed if their dues are received immediately.

This issue will also carry the customary historical information about the ASSOCIATION, the constitution and by-laws, lists of former officers and lists of current committees.

ROBERT P. FISCHELIS,
Secretary



New Policy for Presenting N.N.R. Information

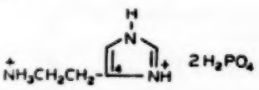
In order to give pharmacists more complete coverage, the important information made available by the A.M.A. Council on Pharmacy and Chemistry is being presented with the cooperation of the Editor of the *Journal of the A.M.A.* in the *Scientific Edition* as well as the *Practical Pharmacy Edition* starting with the April issues of THIS JOURNAL. All A.PH.A. members receive both issues of THIS JOURNAL. Those members who have clipped the N.N.R. monographs in the past will find the arrangement of this material in the *Scientific Edition* suitable for continuing this practice.

Complete N.N.R. monographs will be published in the *Scientific Edition* as soon as they become available. Summary notices of this material with correlating references, will appear in the corresponding issue of the *Practical Pharmacy Edition*.

The *Practical Pharmacy Edition* will also report additional N.N.R. information of immediate value to pharmacists.

N.N.R. MONOGRAPHS

HISTAMINE PHOSPHATE, U. S. P.—The structural formula of histamine phosphate may be represented as follows:



Actions and Uses.—Histamine exists in various organs and tissues of the body, probably in an inert form. It produces local vasodilatation when released from the cell under appropriate stimuli, such as trauma, shock, and possibly allergic reactions. When injected into an animal, histamine stimulates gastric secretion and produces flushing, nausea, bronchospasm, fall in blood pressure, arrhythmia, and gastrointestinal contraction. It acts directly on the receptive substance in smooth muscle.

Histamine, although absorbed orally, produces highly variable effects when administered by this route. Salts of histamine are usually administered subcutaneously, intravenously, or intramuscularly.

Histamine phosphate is employed as a test for gastric secretory activity. It also produces temporary benefit in some patients with Ménierè's syndrome, including those showing sudden deafness. It has been used in the treatment of multiple sclerosis; although the effects are equivocal, they deserve further study. Some patients apparently experience temporary amelioration of the disease after histamine therapy.

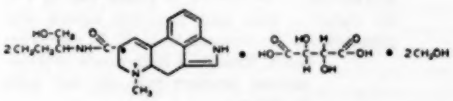
Although histamine has been recommended for treatment of migraine and certain cephalgias, the evidence of value is not convincing. Because of the known hazards of histamine therapy, the drug should not be used indiscriminately in these conditions.

Histamine is a potent drug, and overdosage or administration to susceptible persons may give rise to serious reactions. Vasomotor collapse, shock, and even death may occur quickly if the drug is administered too rapidly or in too great a quantity. Thus, when calculating dosages, it should be remembered that the salt contains only about 36% of the active base. Epinephrine hydrochloride is the antidote of choice in histamine overdosage and should be given intramuscularly or, in severe poisoning, intravenously. A solution of epinephrine hydrochloride 1:1,000 should always be readily available at the time histamine is administered.

Dosage.—For the treatment of Ménierè's syndrome and multiple sclerosis, a slow intravenous injection of histamine phosphate, 1.1 mg. per 100 cc., in isotonic sodium chloride solution may be administered. The initial rate of administration should not exceed 20 to 30 drops per minute and should never exceed 50 to 60 drops per minute. A maximum of 250 cc. of such a solution may be administered in not less than ninety minutes. The therapy may be repeated daily until improvement is noted or until it is determined that the patient will not respond.

Any reaction should be treated immediately with the intramuscular or intravenous injection of 1:1000 epinephrine hydrochloride.

METHYLERGONOVINE TARTRATE.—Methergine Tartrate (Sandoz).—$(C_{20}H_{23}N_3O_2)_2 \cdot C_4H_6O_6 \cdot 2CH_3OH$.—M. W. 893.02.—N-[α-Hydroxymethyl)-propyl]-*d*-lysergamide tartrate containing two molecules of methanol of crystallization.—*d*-Lysergic acid-*d*,*l*-hydroxybutylamide-2 tartrate containing two molecules of methanol of crystallization.—The structural formula of methylergonovine tartrate may be represented as follows:



Actions and Uses.—Methylergonovine tartrate, a partially synthesized derivative of ergonovine maleate, is similar in action to the parent compound and other oxytocic alkaloids of ergot. See *New and Nonofficial Remedies* under the general statement on oxytocics and the monograph on ergonovine maleate.

Methylergonovine tartrate induces uterine contractions in the immediate period after placental expulsion and in the puerperium by either parenteral or oral administration (within thirty to sixty seconds after intravenous injection, two to five minutes after

(Continued on page V)

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N.N.R.

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intramuscular injection, and three to five minutes after oral administration). Clinical observations indicate that the intensity and duration of its oxytocic effect is somewhat greater than that of ergonovine maleate, but less prolonged than that of ergotamine tartrate.

Dosage.—Methylergonovine tartrate is administered orally, intramuscularly, or intravenously. Injection should be used immediately following delivery of the anterior shoulder or the placenta. A single dose of 0.2 mg. is injected intramuscularly or intravenously at the end of labor. If atony and hemorrhage persist post partum, further injections of the same dose may be given at intervals of two to four hours. A dose of 0.2 mg. may be given orally three or four times daily in treating subinvolution or during postpartum convalescence in place of the parenteral route.

Tests and Standards.—

Physical Properties: Methylergonovine tartrate is a white to pinkish tan, odorless, bitter, microcrystalline powder. It is very soluble in water, freely soluble in alcohol, and very slightly soluble in chloroform and in ether. Methylergonovine tartrate must be protected from light and heat. The *p*H of a 0.02% solution is 5.0–5.8.

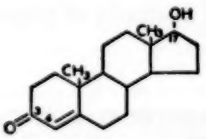
[For more information regarding this monograph see *J. Am. Med. Assoc.*, **154**, 834 (March 6, 1954).]

Sandoz Pharmaceuticals, Division of Sandoz Chemical Works, Inc., New York.

Solution Methergine Tartrate: 1-cc. ampuls. A solution containing 0.2 mg. of methylergonovine tartrate in each cc. (*p*H 3.5–4.5).

Tablets Methergine Tartrate: 0.2 mg. U. S. patent 2,265,207. U. S. trademark 400,893.

TESTOSTERONE, U. S. P.—Androlin (Lincoln).—$C_{19}H_{28}O_2$.—M.W. 288.41.—The structural formula of testosterone may be represented as follows:



Actions, Uses, and Dosage.—Testosterone in aqueous suspension apparently has a slightly lesser intensity and slightly greater duration of androgenic action than testosterone propionate. See monograph on testosterone propionate in N. N. R.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **154**, 1002(March 20, 1954).]

TETRAETHYLAMMONIUM CHLORIDE.—Etamon Chloride (Parke, Davis).—$C_8H_{20}ClN$.—M. W. 165.71.—Tetraethylammonium chloride is made in the form of a 50 per cent solution in water. From this solution the dosage forms are prepared. The structural formula of tetraethylammonium chloride may be represented as follows:

$$(C_2H_5)_4N^+Cl^-$$

Actions and Uses.—Tetraethylammonium chloride is a quaternary ammonium compound belonging to a class of drugs that, like nicotine and curare, act as generalized ganglionic blocking agents. The drug partially blocks transmission of motor nerve impulses through the ganglia of both the sympathetic and parasympathetic divisions of the autonomic nervous system. Its action is not adrenolytic and is interrupted by neostigmine. The blocking of sympathetic stimuli associated with vasospasm usually results in an increased blood supply to the affected region, accompanied by reduction in arterial pressure due to vasodilation. The simultaneous interference with the transmission of parasympathetic impulses produces loss of ocular accommodation, cessation or decrease of motility of the gastrointestinal tract, and alteration of urinary bladder function.

Tetraethylammonium chloride is of limited clinical usefulness as a therapeutic and diagnostic agent in the management of peripheral vascular diseases and other conditions involving vascular dysfunction.

Tetraethylammonium chloride promptly lowers blood pressure in both normal and hypertensive patients. Peripheral circulatory collapse has followed its use. Patients occasionally also experience dyspnea with hyperventilation, weakness, fatigue, lightheadedness, slowing of speech, dulling of the sensorium, difficulty in muscle movement that is not associated with impairment of the deep reflexes, dryness of the mouth, and loss of ocular accommodation. The drug should be employed with caution in patients with severe hypertension, particularly in the presence of poor renal function, or high diastolic blood pressure. Repeated administration should be avoided in the presence of impaired renal excretion. Tetraethylammonium chloride is not suitable for the treatment of hypertension because its hypotensive effect is not sustained. It should not be used in patients with recent coronary thrombosis and should be used with caution in all elderly patients, and those with arteriosclerosis because they often experience unusual decrease in blood pressure with diminution in blood flow through the extremities.

Dosage.—Tetraethylammonium chloride is administered by intravenous or intramuscular injection. Intramuscular injection produces local tenderness and burning. Subcutaneous injection produces considerable local irritation and oral administration is ineffective.

The intravenous dose is 2 to 5 cc. of a solution representing 0.2 to 0.5 Gm. (not to exceed 7 mg. per Kg. of body weight). Frequency of injection depends on duration of relief of symptoms. The effectiveness of the dose can be judged properly only on the basis of three or more injections. Injections may be given once or twice daily for several weeks in exceptional cases. The effects of the drug appear almost immediately after intravenous administration and postural hypotension lasts from several minutes to one hour. Patients should be kept recumbent for at least one hour after intravenous injection.

The intramuscular dose is 10 to 12 cc. (5 to 6 cc. in each buttock) of the same concentration as that used intravenously, representing 1 to 1.2 Gm. (not to exceed 20 mg. per Kg. of body weight). This maintains an effective sympathetic block for six to thirty-six hours in hospitalized patients. Continuation of the autonomic blockade for longer than thirty-six hours usually causes considerable distress. Addition of 1 cc. of 2 per cent procaine hydrochloride

(*Continued on page VII*)

101st Convention

American Pharmaceutical Association

and

Affiliated and Related Organizations

Hotel Statler
Boston, Mass.
August 22-27, 1954

The next annual meeting of the American Pharmaceutical Association will be held in Boston, Mass., August 22 to 27, 1954.

Members planning to present papers before any Section during this meeting are earnestly requested and strongly urged to notify the appropriate Section secretary of their intention promptly.

Titles and abstracts *(*not to exceed 200 words*)* of papers should be submitted by *May 15, 1954, or preferably earlier,* in order to insure a place on any section program. Send titles and abstracts of papers to the appropriate Section Secretary.

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For detailed information address the

Secretary of the American Pharmaceutical Association, 2215 Constitution Avenue, N. W., Washington 7, D. C.

N.N.R.

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solution to the dose of tetraethylammonium chloride decreases the discomfort caused by intramuscular injection.

Peripheral circulatory collapse should be treated by artificial respiration and/or injection of epinephrine hydrochloride solution 1:1,000. Intravenous administration of 0.5 to 1 mg. of neostigmine methylsulfate in solution antagonizes the blocking action of tetraethylammonium chloride and promotes rapid recovery from the postural hypotension.

Tests and Standards.—

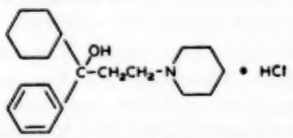
Physical Properties: Tetraethylammonium chloride, isolated by evaporating the 50% solution in a vacuum, is an extremely hygroscopic, odorless, white solid. It is very soluble in water and in alcohol, freely soluble in chloroform, and practically insoluble in benzene and in ether. The *p*H of the 50% and 10% solutions is 5.8 to 6.5.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **154,** 506(Feb. 6, 1954).]

Parke, Davis & Company, Detroit.

Solution Etamon Chloride 10%: 20-cc. Steri-Vials. A solution containing 0.1 Gm. of tetraethylammonium chloride in each cc. Preserved with 0.005% benzethonium chloride. U. S. trademark 432,476.

TRIHEXYPHENIDYL HYDROCHLORIDE.—Artane Hydrochloride (Lederle).—$C_{20}H_{31}NO.HCl$.—M.W. 337.92.—3-(1-Piperidyl)-1-phenyl-1-cyclohexyl-1-propanol hydrochloride.—The structural formula of trihexyphenidyl hydrochloride may be represented as follows:



Actions and Uses.—Trihexyphenidyl hydrochloride, a synthetic piperidyl compound, exhibits an atropine-like action and exerts an antispasmodic effect on smooth muscle and an inhibitory action on the parasympathetic nervous system. Thus, spasm of smooth muscle is relieved by direct action and by indirect parasympathetic release. The drug also relieves spasticity of voluntary muscle, partly because of its parasympatholytic action and partly because of action on the cerebral motor centers. Unlike atropine, the action of trihexyphenidyl is strongest in producing desirable, relaxant effects, and weakest in producing undesirable side-effects. Compared with atropine, trihexyphenidyl produces about one-half as intense antispasmodic action, one-third as great mydriatic action, one-eighth as much antisialogogue effect, and one-tenth as much cardiovagal inhibition.

Trihexyphenidyl hydrochloride is useful for the treatment of all forms of parkinsonism, including the postencephalitic, arteriosclerotic, and idiopathic types. It reduces the muscular rigidity and relieves the depression and mental inertia characteristic of this syndrome. The drug is especially effective in reducing the rigidity produced by muscle spasm, thus increasing the ability of the patient to achieve coordination of muscular motions. Tremor is usually reduced, but in some patients who have been severely spastic, it may become more perceptible as spasticity is relieved. Sialorrhea is reduced, but with less accompanying mouth dryness, blurred vision, or mydriasis than with the use of atropine. Trihexyphenidyl is particularly useful in the treatment of arteriosclerotic parkinsonism because, unlike atropine, it usually does not tend to precipitate glaucoma.

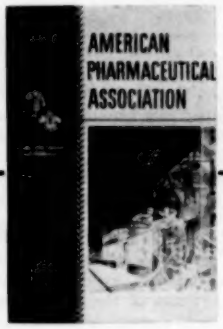
Trihexyphenidyl hydrochloride thus far has seldom produced severe reactions with therapeutic dosages. Some patients experience minor side-effects such as dryness of the mouth, blurring of vision, dizziness, mild nausea, or nervousness. Compared with the symptoms of unrelieved parkinsonism, they are usually not troublesome and may be controlled by adjustment of dosage or time of administration. These side-effects tend to decrease with continued use of the drug. The infrequent but more severe reactions of mental confusion, agitation, or nausea with vomiting tend to occur in arteriosclerotic patients or in persons exhibiting other drug idiosyncrasies. Such patients must develop tolerance to the drug gradually, beginning with a smaller initial dose and increasing the dosage more slowly until an effective level is reached. If a severe reaction occurs, administration of the drug should be discontinued temporarily for several days and then resumed at a lower dosage level. Hypertension or cardiac, liver, or kidney disorders do not contraindicate use of the drug, but patients with these conditions should be carefully observed.

Dosage.—Trihexyphenidyl hydrochloride is administered orally. The usual initial dose is 1 mg. for the first day. If the patient is already receiving treatment with other agents, this initial dose should be substituted for a part of the current therapy. As the dosage of trihexyphenidyl is gradually increased, other medication should be decreased until the drug has replaced the former treatment or until an effective balance has been achieved. With prior therapy and in arteriosclerotic or sensitive patients, daily increments of the dose should be small until satisfactory tolerance is attained. If prior medication or unusual reactivity is not involved, the dosage is increased to 2 mg. for the second day, with subsequent increments of 2 mg. daily until a total daily amount of 6 to 10 mg. is reached. Postencephalitic patients may require as much as 12 to 15 mg. daily. At the lower level of daily dosage, the total amount can be divided into three equal parts, taken near meal times; at the higher level, a fourth dose at bedtime is required. Patients are allowed to choose whether to take the medication before or after meals. Postencephalitic patients, who have more excessive salivation, will prefer administration after meals and may require small doses of atropine sulfate as an adjuvant. Whenever the mouth becomes excessively dry, the drug can be taken before meals unless this causes nausea; if it is necessary to administer the dose after meals, dryness can be allayed by hard candy, gum, or extra intake of fluid.

Tests and Standards.—

Physical Properties: Trihexyphenidyl hydrochloride is a white, odorless solid, m. p. 249.0–249.5° (with slight decomposition). It is freely soluble in methanol, and very slightly soluble in ether and in benzene. The approximate amounts which dissolve at 25° in the following solvents to form 100 ml. of solution are: 6 Gm. in alcohol, 5 Gm. in chloroform, and 1 Gm. in water. The *p*H of a 1% solution is 5.5–6.0.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **154,** 1001(March 20, 1954).]



A Preview of Advertising

In the May 1954 issue of the Practical Pharmacy Edition

(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

▶ Featured by **Abbott Laboratories** are **Iberol**—"Iron-Plus"— and their antibiotic, **Erythrocin Stearate.**

▶ **Span, Tween, Arlacel,** and **Sorbo** are some of the adjuncts offered by **Atlas Powder Company** for use in pharmaceutical preparations.

▶ **Ciba** announces that new fair trade minimums and quantity discounts make a 40 per cent profit possible on its entire line.

▶ **Ilidar Phosphate,** for vasospasm, and **Gantrisin Cream,** for vaginal use, are introduced by **Hoffmann-La Roche.**

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▶ It's "top promotion time for these **Pfizer** profit makers: new long-acting **Bonamine** and **Terramycin Intramuscular."**

▶ Because of its excellent reputation, **Philadelphia College of Pharmacy and Science** attracts students from many distant states and nations.

▶ **Reed & Carnrick** introduces **Lullamin Drops,** nonbarbiturate sedative for pediatric use.

▶ "Now take the topical route to greater **Tetracyn** profits!" advocates **J. B. Roerig.**

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▶ **Phenergan** for the symptomatic relief of pollen hay fever is publicized by **Wyeth.**

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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLIII — *MAY, 1954* — NUMBER 5 — CONSECUTIVE NO. 10

Studies in Ergot*

II. Alkaloids of North American Ergot

By GORDON H. SVOBODA, GEORGE SHAHOVSKOY, A. THORNTON OLIVER, EROLD DILLER, FLORENCE BARTON, and ALBERT J. BARNES, Jr.†

A phytochemical investigation of North American ergot was undertaken, using a modification of the extraction procedure applied previously to ergotamine-containing Swiss ergot. The peptides isolated as phthalates consisted of ergotamine and ergocristine apparently in solid solution. Rye ergots of Canadian and Japanese origins gave the same results, as did American barley and durum wheat ergots. All of these ergots also yielded ergonovine, and thus for the first time isolation of all three types of alkaloids, i. e., ergotoxine, ergotamine, and ergonovine from the same ergot is reported. The mixed ergotamine-ergocristine salt was separated into its component parts. Valuable physical data for the various compounds are reported.

IT HAS BEEN ESTABLISHED that ergotamine can be readily isolated from Central European ergot as the phthalate salt (1), and it was the purpose of this investigation to extend this technique to rye ergot from other sources, particularly from the United States. The domestic ergots used in this study included those from rye, durum wheat, and barley of Minnesota and neighboring States.

EXPERIMENTAL DESIGN

The procedure represented a departure from that used in the extraction of the Central European ergot. The use of an acid reagent, such as $Al_2(SO_4)_3 \cdot 18H_2O$ (2), to bind the alkaloids in the drug during defatting was found to be unnecessary. The ground ergot could be reasonably well defatted with extraction naphtha, and while not as much fatty material was removed as with benzene, no difficulties were encountered in the processing by the phthalate procedure. This procedure can presumably be extended to the extraction of any ergotamine containing ergot with equal success.

The crystalline phthalate isolated from domestic ergot appeared to be a mixture of ergotamine and ergocristine phthalates in solid solution. Acid hydrolysis studies of this salt showed the presence of phenylalanine and proline among the reaction products, while alkaline hydrolysis gave pyruvic and dimethylpyruvic acids. The hydrolysis products of the known peptide alkaloids of ergot are given in Table I.

Application of a paper chromatographic method developed by Bird (4) appeared to further substan-

TABLE I.—DEGRADATION FRAGMENTS OF PEPTIDE ALKALOIDS OF ERGOT (3)

Ergosine	L-leucine, pyruvic acid, D-proline, NH_3, lysergic acid
Ergotamine	L-phenylalanine, pyruvic acid, D-proline, NH_3, lysergic acid
Ergocornine	L-valine, dimethylpyruvic acid, D-proline, NH_3, lysergic acid
Ergocryptine	L-leucine, dimethylpyruvic acid, D-proline, NH_3, lysergic acid
Ergocristine	L-phenylalanine, dimethylpyruvic acid, D-proline, NH_3, lysergic acid

* Received January 6, 1953, from the Lilly Development and Control Laboratories, Eli Lilly and Company, Indianapolis, Ind.

† The authors are grateful to Drs. S. F. Kern and H. A. Rose for their X-ray and crystallographic studies, Dr. H. E. Boaz for the infrared absorption spectrum, Mr. D. O. Woolf, Jr., for electrometric titrations, Messrs. J. M. Goodyear, H. L. Hunter, and G. M. Maciak for the analytical data, Mr. H. L. Bird, Jr., for chromatographic studies, and Dr. Y. Loo for hydrolysis studies.

tiate the above conclusion. This method involved the running of simultaneous strips of phthalate and authentic alkaloids, using formamide as the stationary phase and formamide-saturated benzene as the mobile phase. R_f values of the two-spot phthalate were identical with those of authentic ergotamine and ergocristine, run both as separate entities and as an artificially prepared mixture.

Thus having established the identity of the two alkaloids, it remained to determine the absolute amounts of each in the mixture. X-ray measurements, crystallographic studies, and rotational data indicated that a mixture of varying composition was involved. Application of countercurrent analysis of mixed and authentic phthalates in a system of ethyl acetate-10% acetic acid confirmed this.

One mixed phthalate thus isolated crystallized from acetone as transparent rhombic plates, Fig. 1, with a profile angle of 83°; $n_1 = 1.592$, $n_2 = 1.530$ (27°, sodium light), m. p. 178° (decomp.), $[\alpha]_D^{25}$ + 68° (1% in methanol or 90% acetone) and $[\alpha]_D^{25}$ − 158° (1% free base in chloroform).

X-ray diffraction data on this compound, Table II, were obtained using a Norelco 114.59-mm. diameter camera and vanadium oxide filtered chromium radiation ($\lambda = 2.2850$ A.). The sample was mounted in a parlodion capillary having an inside diameter of 0.4 mm.

A typical elemental analysis gave the following results: C = 64.10%, H = 5.34%, N = 8.98%, when the sample was dried *in vacuo* for twenty-four hours at 60°.

Electrometric titration of this phthalate in 33% dimethyl formamide showed three groups with the following pK'_a values: 3.64, 6.23, 7.0. Independent titration of phthalic acid gave pK'_a values of 3.7 and 7.0. This phthalate was evidently composed of one molecule of base and one molecule of acid. Its apparent molecular weight was 780 ± 30.

Fig. 1.—Typical phthalate crystals.

TABLE II.—X-RAY DIFFRACTION DATA FOR A MIXED PHTHALATE

d	I/I_1	d	I/I_1
13.9	3.0	5.23	3.0
13.0	0.1	4.97	0.6
11.9	3.0	4.39	0.4
10.8	0.6	4.18	0.1
9.51	0.8	4.04	0.1
9.00	1.0	3.94	0.1
8.31	0.2	3.85	0.1
7.36	0.1	3.73	0.8
6.54	0.6	3.63	0.4
6.34	0.8	3.48	0.2
6.15	0.6	3.30	0.1
6.00	0.2	3.11	0.1
5.76	0.1	3.02	0.1
5.43	0.1	2.80	0.1
		2.74	0.1

The infrared absorption spectrum of this sample of phthalate is included in Fig. 2.

The free base was readily isolated from the salt and recrystallized from benzene as transparent *o*-rhombic prisms. Single crystal studies produced the following data:

Crystal system: ortho-rhombic.
Form and habit: Rods and prisms from benzene showing the macropinacoid (100), prism (110), and macrodone (101).
Cell dimensions:
$A_0 = 15.92 \pm 0.09$ *A*, $B_0 = 14.44 \pm 0.07$ *A*,
$C_0 = 18.26 \pm 0.09$ *A*.
Formula weights per cell: 4.
Formula weight: 601.2 ± 12 (calculated from cell dimensions and density)
Density: 1.199 Gm./cc. (flotation)
Refractive indices:
$\alpha = 1.54$ (calc. from 2 *V*, β and r),
$\beta = 1.616 \pm 0.003$, $\lambda = 1.635 \pm 0.003$
Optical axial angles: $2V = 65° \pm 3°$
Dispersion: $V > R$
Optic axial plane: 001
Sign of double refraction: Negative
Acute bisectrix: $\alpha = a$

The value of the molecular weight found in this work (601.2) lay between those of ergotamine (581) and ergocristine (609), but was closer to the latter. The value found for the optic axial angle (65°) was between those for ergotamine (56°) and ergocristine (74°). The literature values (5) of the refractive indices of ergocristine are uncertain and lie too close to those given for ergotamine to be of much value in differentiation.

The mixed phthalate was separated into its pure component parts by fractional crystallization of the free base and crystallization as the ethanesulfonate, but only at a considerable sacrifice of starting material. The ergotamine ethanesulfonate crystallized from acetone as large rhombic plates having parallel extinction, positive elongation and a profile angle of 85°; $n_1 = 1.645$, $n_2 = 1.545$. This salt crystallized from ethanol as rhombic plates with sym-

metrical extinction and negative elongation, n_1 = 1.600, n_2 = 1.520. The crystal edges were rounded so that a profile angle would be subject to large error. The phthalate prepared from the free base crystallized from acetone as blades having parallel extinction, positive elongation, and a profile angle of 82°; n_1 = 1.630, n_2 = 1.545. The ergocristine phthalate crystallized from acetone as blades having parallel extinction, positive elongation, and a profile angle of 82°; n_1 = 1.580, n_2 = 1.510. Comparisons were made with corresponding salts prepared from authentic samples of ergotamine and ergocristine,[1] as well as with salts of ergotamine isolated from cultivated Swiss ergot (1), with the results as set out in Table III.

X-ray data for various ergotamine and ergocristine derivatives are given in Table IV.

Rye ergot, other than domestic, was investigated and included Canadian, cultivated Japanese, and Rumanian samples. Two other domestic ergots were included in this study, those from durum wheat and barley. Data on the mixed phthalate yields are given in Table V. While not within the scope of this paper, it may be mentioned that ergonovine was readily isolated as the maleate from all of these samples, including the Swiss ergot previously investigated (1).

None of the samples of Spanish commercial ergot examined yielded any crystalline phthalate. We are in direct agreement with Smith and Timmis (6) in that the source of ergot determines the alkaloid content rather than the so-called "protective" method of isolation of Stoll and Hofmann.

EXPERIMENTAL PROCEDURE

Extraction Procedure.—Forty-five hundred grams of American rye ergot were carefully ground through a No. 12 screen, the mill being chilled during this operation, as with solid CO_2. The drug was then mixed with 850 cc. of 11% ammonium hydroxide and charged into a jacketed percolator, the temperature of which was maintained at 15–20° throughout the course of percolation.

The drug was defatted with 17.5 L. of extraction naphtha, approximately 11% of the drug weight being obtained as a viscous yellow oil. Further percolation of the drug with 25 L. of thiophene-free benzene resulted in the extraction of the peptide alkaloids. Concentration *in vacuo* of the benzene percolate to 1,500 cc., with the subsequent addition of 7,500 cc. of extraction naphtha, yielded 17.5 Gm. of a gray amorphous base. The crude base was extracted with ether, and 10.4 Gm. of amorphous phthalate was precipitated by the addition of an ether solution containing 4 Gm. of phthalic acid. Crystallization and recrystallization from acetone yielded 4.97 Gm. of crystalline phthalate.

Separation of Mixed Phthalate.—Ten grams of phthalate were suspended in 5 L. of water. The mixture was made alkaline to *p*H 9 with ammonium hydroxide and extracted with 2–10 L. and 2–5 L. portions of thiophene-free benzene. After being dried, the extract was concentrated *in vacuo* until crystallization began. The first crop of base (I) was filtered, washed, and dried *in vacuo* for twelve hours at 40° (wt. = 4.2 Gm.). The mother liquor was concentrated *in vacuo* until crystallization began and finally to a thick syrup, yielding 3.16 Gm. (II) and 1.84 Gm. (III) respectively. The bulk of the ergotamine was found in (I), while (II) and (III) were composed essentially of ergocristine.

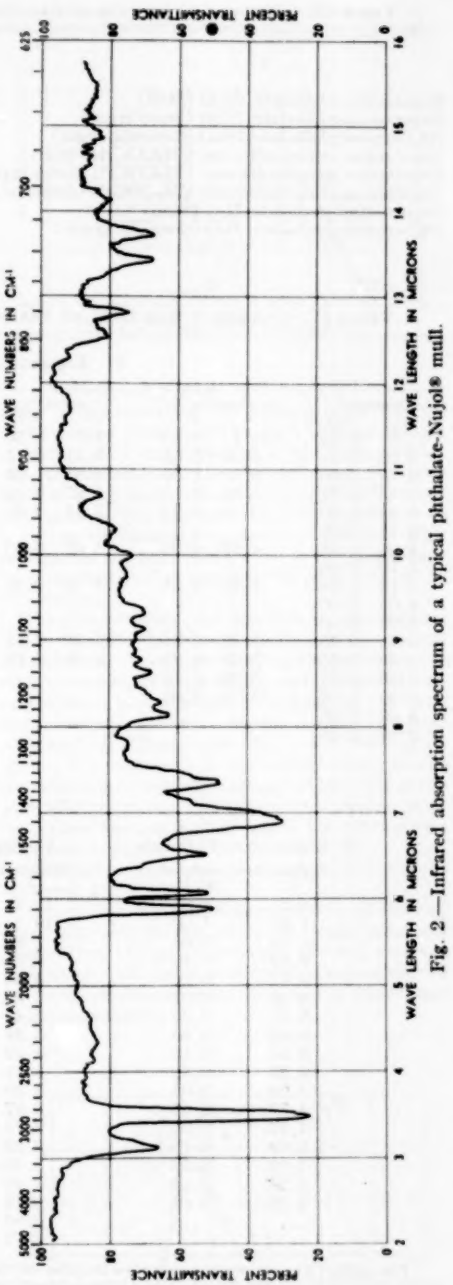

Fig. 2.—Infrared absorption spectrum of a typical phthalate-Nujol® mull.

[1] Kindly supplied by Dr. A. E. Stoll, Sandoz.

TABLE III.—PHYSICAL CONSTANTS OF ERGOTAMINE AND ERGOCRISTINE FROM SEVERAL SOURCES

	$[\alpha]_D^{25}$ (1% Base in $CHCl_3$)	N (Calcd.)	N (Found)
Ergotamine phthalate.$2H_2O$ (Stoll)	−140	8.93	9.12
Ergotamine phthalate.$2H_2O$ (Swiss ergot)	−143	8.93	9.03
Ergotamine phthalate.$2H_2O$ (domestic ergot)	−141	8.93	9.03
Ergotamine ethanesulfonate.CH_3COCH_3 (Stoll)	−140	9.35	9.36
Ergotamine ethanesulfonate.CH_3COCH_3 (Swiss ergot)	−144	9.35	9.58
Ergotamine ethanesulfonate.CH_3COCH_3 (domestic ergot)	−143	9.35	9.37
Ergocristine phthalate.H_2O (Stoll)	−170	8.82	8.84
Ergocristine phthalate.H_2O (domestic ergot)	−163	8.82	8.72

TABLE IV.—POWDER X-RAY DATA ON VARIOUS ERGOTAMINE AND ERGOCRISTINE DERIVATIVES

A. Ergotamine Ethanesulfonate

Acetone Recrystallized								Ethanol Recrystallized			
Domestic[a]		Domestic		Swiss		Stoll		Domestic		Stoll	
d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1
11.6	0.63	11.7	0.50	11.7	0.50	11.7	0.67	12.4	1.00	12.4	1.00
8.29	0.63	8.39	0.62	8.31	0.75	8.36	0.67	11.2	0.05	11.2	0.06
6.78	1.00	6.81	1.00	6.81	1.00	6.81	1.00	10.3	0.05	10.6	0.06
5.85	0.13	5.88	0.13	5.87	0.13	5.92	0.17	9.58	0.05	9.79	0.06
5.44	0.50	5.43	0.50	5.45	0.50	5.46	0.67	8.49	0.30	8.52	0.38
5.10	0.06	...	..	...	..	...	..	7.95	0.05	8.01	0.06
4.96	0.38	4.97	0.50	5.01	0.50	4.98	0.17	7.40	0.20	7.43	0.25
4.70	0.06	...	..	...	..	...	..	6.60	0.20	6.61	0.20
4.18	0.38	4.20	0.25	4.18	0.25	4.19	0.17	6.22	0.25	6.23	0.25
4.05	0.06	...	..	...	..	...	..	5.77	0.10	5.83	0.25
3.93	0.06	...	..	...	..	...	..	5.29	1.00	5.30	1.00
3.83	0.25	3.82	0.25	3.82	0.13	3.80	0.17	4.94	0.10	4.96	0.13
3.59	0.25	3.60	0.25	3.61	0.13	3.59	0.33	...	..	4.46	0.06
3.50	0.13	3.49	0.13	...	..	...	..	4.39	0.05	4.37	0.06
3.30	0.06	3.30	0.06	...	..	...	..	4.17	0.05	...	..
3.09	0.06	...	..	...	..	...	..	3.96	0.05	3.99	0.06
2.99	0.06	...	..	...	..	...	..	...	..	3.86	0.06
								3.71	0.20	3.71	0.25
								3.49	0.10	3.50	0.06
								3.41	0.05	3.42	0.06
								3.17	0.05	...	..

B. Ergocristine Phthalate

Acetone Recrystallized	
d	I/I_1
14.2	1.00
11.85	1.00
10.95	0.05
9.62	0.10
9.12	0.20
8.26	0.05
6.47	0.20
6.25	0.20
6.05	0.10
5.25	0.80
4.98	0.05
4.37	0.05
4.17	0.05
3.69	0.10
3.49	0.05
3.28	0.05
3.13	0.05
...	..
...	..

C. Ergotaminine

Methanol Recrystallized	
d	I/I_1
13.8	0.63
10.5	0.06
7.88	1.00
6.81	1.00
5.88	0.50
5.62	0.25
5.43	0.25
5.24	0.50
4.59	0.38
4.31	0.25
4.10	0.13
3.87	0.06
3.72	0.06
3.59	0.06
3.48	0.06
3.40	0.06
3.19	0.06
2.94	0.06
2.72	0.06

D. Ergocristinine

Methanol Recrystallized	
d	I/I_1
14.4	0.25
12.9	0.38
10.3	0.13
8.79	0.13
8.26	1.00
7.66	0.06
7.26	0.13
6.58	0.13
6.39	0.13
5.46	0.63
4.65	0.25
4.47	0.06
4.27	0.06
4.18	0.06
4.03	0.06
3.68	0.06
3.59	0.06
...	..
...	..

[a] This pattern was somewhat sharper than the others of the acetone group, thus accounting for the greater number of measurable lines. The salt was recrystallized 3 times from acetone, resulting in a somewhat higher degree of crystallization.

A 1.25-Gm. sample of (I) was dissolved in 4 cc. of acetone, and 5 cc. of 0.5 *N* ethanesulfonic acid was added, producing an immediate clouding and deposition of a gummy mass on the walls of the flask. Dilution with acetone to 50 cc. and warming to 40° caused complete solution. Slow cooling to room temperature induced crystallization. A light gray-green crystalline material was collected (wt. =

TABLE V.—MIXED PHTHALATE YIELD DATA ON 10-LB. LOTS

	Rye, Gm.	Durum Wheat, Gm.	Barley, Gm.
Domestic	4.97	2.6	4.4
Canadian	3.53	...	...
Japanese	3.50	...	...
Rumanian	0.11	...	...

0.45 Gm.). Recrystallization from acetone yielded 0.4 Gm. of chromatographically pure ergotamine ethanesulfonate. This salt was also readily prepared in an alcoholic medium.

A 1.25-Gm. sample of (III) was dissolved in ether, and an ether solution containing 0.4 Gm. of phthalic acid was added, yielding 1.31 Gm. of amorphous phthalate. Crystallization and recrystallization from acetone produced 0.57 Gm. of first crop and 0.57 Gm. of second crop material, the latter being chromatographically pure ergocristine phthalate.

Ergotaminine and Ergocristinine.—As further proof of identity the L-forms were converted to the D-forms. A 0.5-Gm. sample of the respective L-base was dissolved in 25 cc. of methanol, which contained 3 drops of glacial acetic acid. The solution was boiled for ten minutes and allowed to stand overnight at room temperature. Although ergotaminine was more methanol-insoluble than ergocristinine, it required a much longer period of heating to effect its conversion.

Countercurrent Extraction.—The countercurrent distribution was run in a 60-tube Craig apparatus (H. W. Post Co.) which required 100 ml. per phase, using the system of redistilled ethyl acetate–10% acetic acid. The system was equilibrated before use. The distribution was run in a dark, constant-temperature room of 25°. A load of 80–100 mg. was found to be the maximum for successful distribution without the complication of emulsion formation. A total of 30 inversions for each transfer was made to effect equilibration of the alkaloid between the two phases.

Upon completion of the distribution the two phases were removed from each tube and stored in the dark and cold until further processing. To make a determination the ethyl acetate was removed under vacuum in the dark without the aid of heat. With this technique a minimum destruction of the alkaloid was obtained, and the total alkaloid in a tube was concentrated in the 10% acetic acid phase. The solution was then transferred to a volumetric flask and made up to 100 ml. This represented the total alkaloid for an individual tube of a distribution and was done for each tube in the distribution.

The determination was based on the fluorescence of the alkaloid, this being a function of the lysergic acid nucleus. It was found to be a very sensitive and simple method for determining such alkaloidal concentration. Measurements were made on the Coleman photofluorometer, Model I-110. Readings were made at 365 mμ, with the use of a Woods glass filter. The sample to be determined was prepared by making an appropriate aliquot of the 10% acetic acid solution of the alkaloid up to a volume of 10 ml. Care was exercised to expose the solution to a minimum of light.

Calculations were based on standard curves of the alkaloids, Fig. 3.

To establish the partition coefficients of the two ergot alkaloids of interest, i. e., ergotamine and ergocristine, known preparations of each were run separately. The peaks were qualitatively confirmed by paper chromatography, both in the known and unknown mixtures.

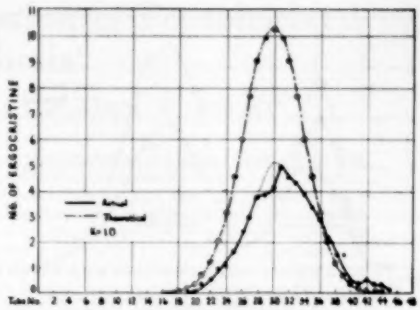

Fig. 4.—Ergocristine distribution.

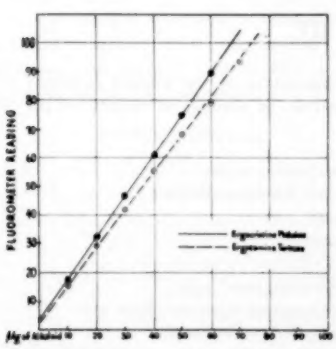

Fig. 3.—Standard curves of the alkaloids for fluorometric determination.

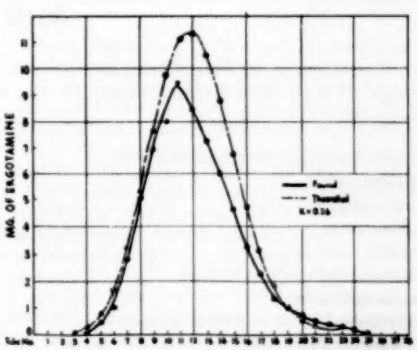

Fig. 5.—Ergotamine distribution.

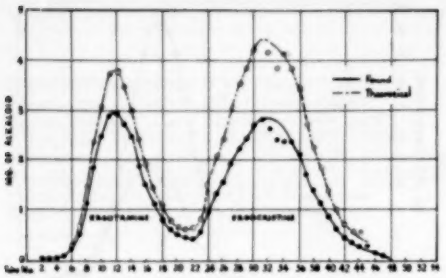

Fig. 6.—Distribution of mixed phthalate.

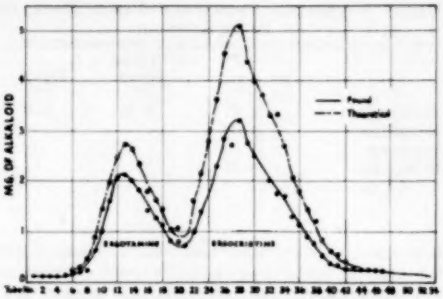

Fig. 7.—Distribution of mixed phthalate.

Figure 4 shows a typical distribution curve of ergocristine of our own preparation, which was obtained in the system and under the conditions described. The ergocristine moved in a homogeneous peak centered on tube 31 and gave a partition coefficient of 1. A recovery of 63% suggested destruction of alkaloid during distribution. Fluorometer analysis of the entire distribution did not reveal any fluorescent material, suggesting that the lysergic acid nucleus was disrupted in some manner during distribution.

The ergotamine sample in Fig. 5 again demonstrated that the distribution was homogeneous, and the partition coefficient was 0.26. A 78% recovery of the ergotamine was realized, which may suggest that it was more stable under operating conditions than was the ergocristine.

The patterns for a mixed phthalate are shown in Figs. 6 and 7. Paper chromatography of the peak materials demonstrated ergotamine and ergocristine as pure components. Calculations of the partition coefficients further showed the similarity of the peaks to their known values.

From the curves presented, it may be concluded that the samples investigated were composed of 1 part ergotamine and 1.0–1.5 parts ergocristine. If corrections were made for the destruction of each alkaloid as calculated from the standard distribution curves, the ratio of the mixture could be calculated as 1 part ergotamine and 1.8–2.0 parts of ergocristine.

SUMMARY

1. The phthalate procedure for ergotamine was extended to the investigation of American ergot and resulted in the isolation of ergotamine and ergocristine phthalates in solid solution of varying composition.

2. Ergonovine was also isolated as the maleate salt.

3. American ergot (rye, durum wheat, and barley) was unique and differed from the European ergot of commerce in that it contained all three classes of ergot alkaloids, i. e., ergotoxine (ergocristine), ergotamine, and ergonovine.

4. This was also found to be the case for the samples of Canadian and cultivated Japanese ergot examined.

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WHO MAKES IT?

The Registry of Rare Chemicals, Armour Research Foundation of the Illinois Institute of Technology, 35 West 33rd Street, Chicago 16, Ill., seeks information on sources of supply of the following chemicals:

Trimethylenediaminetetraacetic acid
Thioglucose
Sedoheptulose
D-Rhamnose
Ptyalin
3,5-Pyridinedicarboxylic acid
Lactic aldehyde
8-Hydroxy-1,2,3,4-tetrahydroquinoline
1-Decanesulfonic acid
beta-Chlorolactic acid
Pseudocholinesterase
2-Methyl-8-aminoquinoline
Hedonal
Agmantine
3,3,3-Trifluoro-1-propene
n-Dodecanesulfonic acid
Tetrachlorohydroquinone diacetate
2-Nitro-4,5-dimethylaniline
9-Decenoic acid
Dimethyl diglycollate

Antihistamine Potentiation of Pentobarbital Anesthesia*

By HAROLD LIGHTSTONE† and JOHN W. NELSON‡

The results of this investigation indicate that the common antihistamine drugs significantly prolong the sleeping time of pentobarbital anesthesia in the rat. The results of liver analyses indicate that the antihistamines do not seem to interfere with detoxication of pentobarbital. Evidence is presented to support the assumption that the antihistamines cause an increase in the rate of entrance of pentobarbital into the brain. The results also indicate that there is a required threshold concentration of pentobarbital in the blood necessary before the antihistamines produce an altered response. The mechanism by which the antihistamines prolong the sleeping time of pentobarbital is apparently unrelated to their common side actions.

THE COMMON SIDE ACTIONS of the antihistamine drugs have been extensively summarized in numerous reports (1–8). Both depression and stimulation of the central nervous system have been observed after administration of the antihistamines (9). The most extensive report on the toxic actions of the antihistamines was by Wyngaarden and Seevers (10). In an attempt to estimate the potential central nervous system depressant action of the antihistamines Winter suggested the administration of a drug of known sedative action and observing the effect of an antihistamine superimposed upon it. He subsequently reported a potentiating effect upon Evipal® (hexobarbital) anesthesia (40–100 mg./Kg.) by using Benadryl®, Pyribenzamine® and Neo-Antergan® (10–20 mg./Kg.) prior to the injection of barbiturate (11). This same phenomenon of potentiation of barbiturate anesthesia was reported by Heinrich (12). However, Cronheim and Ehrlich (13) reported an absence of any potentiating effect using four antihistamines related to Pyribenzamine®. The latest evidence to support the earlier work has been reported by Ambrus, *et al.* (14), who found certain antihistamines potentiated the effect of Evipal® in mice. It is the purpose of this paper to report results obtained by the use of five common antihistaminics in an attempt to demonstrate potentiation of pentobarbital narcosis in rats, and to offer a possible explanation for such a phenomenon.

EXPERIMENTAL

Potentiation.—Wistar strain rats obtained from Carworth farms were used throughout the investigation. In most experiments they were employed in groups of twelve, selected to make the average weight of the control group correspond as closely as possible to the average weight of the experimental animals. In all experiments the rats were fasted for eighteen to twenty-four hours prior to use. The antihistamine drug solutions were prepared in distilled water so as to contain 10 mg./cc. and were not utilized if older than twenty-four hours. The pentobarbital used in this investigation was Abbott's Veterinary Nembutal®, containing 60 mg./cc. Where the effect of the antihistamine upon the recovery time of rats from narcosis induced with pentobarbital was to be observed, the rats received 10 mg./Kg. of the antihistamine subcutaneously, followed in thirty minutes by 60 mg./Kg. of pentobarbital administered intraperitoneally. The time from the loss of the righting reflex to return of the reflex was taken as the duration of sleep. The righting reflex normally returns after the following signs occur: yawning, stretching, movements of the vibrissae, urination, and erection of the tail. In some cases the rats served as their own controls and when such procedure was adopted at least four days was allowed to lapse between experiments. Diphenhydramine hydrochloride (Benadryl®), β-(4-bromobenzhydryloxy)-ethyl-dimethylamine hydrochloride (Ambodryl®), tripelennamine hydrochloride (Pyribenzamine®), phenindamine tartrate (Thephorin®), and chlorphenamine maleate (Chlor-Trimeton®) were used and the doses always refer to the salts of these compounds. Ambodryl® is a bromine analog of Benadryl® and is said to be less depressant in its side action when employed clinically.

Tissue Studies.—All tissues were analyzed for pentobarbital by the method of Goldbaum (15). In the liver analyses twelve rats were used for controls and twelve rats for experimentals. In the case of the brain analyses fifteen rats were used in the experimental study and twelve served as controls. Three rats were used for each time period and analyses were made on individual livers and brains so that three separate readings could be compared. In the experiments comprising the tissue studies the rats received 30 mg./Kg. of pentobarbital instead of the usual 60 mg./Kg. The animals were sacrificed at specified time intervals and tissues immediately removed and placed in vessels at a temperature of zero degrees centigrade. They were analyzed for pentobarbital no longer than one hour later.

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Part of a thesis presented to the Graduate School, The Ohio State University, in partial fulfillment of the requirements for the degree of Master of Science.
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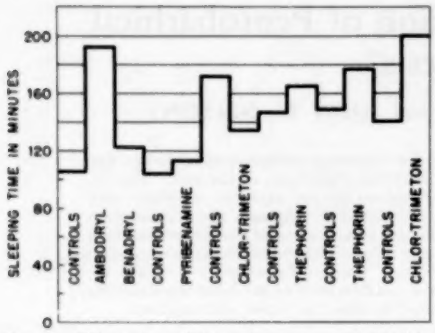

Fig. 1.—Effect of antihistamines on pentobarbital anesthesia using separate control groups.

STATISTICAL

	1	2	3	4	5	6	7
SI	34.23	1.03	3.16	8.25	16.4	10.5	12.
t	0.29	1.65	1.21	1.42	1.18	2.07	3.9
p	>0.5	0.12	0.25	<0.2	<0.3	<0.1	<0.1

RESULTS AND DISCUSSION

Synergism Between Antihistamines and Pentobarbital Using Separate Control Groups.—In a series of six experiments the effects of Benadryl®, Ambodryl®, Pyribenzamine®, Chlor-Trimeton®, and Thephorin® on the sedative action of pentobarbital was studied. In Fig. 1 the sleeping times of the rats given both antihistamine and pentobarbital can be compared to the control group which received only pentobarbital. In all cases but one (Chlor-Trimeton®) the experimental animals slept longer than the controls. It should be pointed out however, that the controls in this particular case had received an antihistamine agent five days earlier.

TABLE I.—EFFECT OF ANTIHISTAMINES ON SLEEPING TIME OF PENTOBARBITAL IN RATS SERVING AS OWN CONTROLS

	—Sleeping Times in Minutes—					
	P[a]	H[b]	P	H	P	H
Controls	184	185	152	132	135	120
Benadryl®	116	145	145	190	75	121
Ambodryl®	138	168	171	224	101	147
Pyribenzamine®	136	161	188	173	93	97
Thephorin®	108	139	123	151	84	81
Chlor-Trimeton®	108	165	141	170	92	103

[a] P—pentobarbital alone.
[b] H—pentobarbital plus antihistamine.
The controls received pentobarbital alone throughout the experiment.

Since it is generally agreed among various investigators that the antihistamines are released slowly from the body and hardly eliminated within five days (16), it is a possibility that the synergistic action was still present even after five days.

Synergism Between Antihistamines and Pentobarbital in Rats Serving as Their Own Controls.—Since the sleeping times of two groups of rats in the same weight range under pentobarbital anesthesia may vary considerably, it was deemed necessary to observe what happens when rats serve as their own controls and receive pentobarbital alone and in combination with antihistamines on alternate occasions.

Five groups of rats were selected to be used with the antihistamines and another group to serve as controls. The control group was run to observe what effect the pentobarbital alone produced on successive administrations. The average weights of the five groups were within 5 Gm. of each other. The sleeping times are recorded in Table I. It will be noted in all cases but two that the sleeping times of rats receiving antihistamines plus pentobarbital were longer than those of rats receiving pentobarbital alone.

The control group showed an almost progressive decline in sleeping times. In Table II the statistical evaluation for all the groups is shown. The probability of the results occurring by chance is less than when the sleeping times were compared to those of separate control groups.

Determination Whether the Antihistamines Cause a True Potentiation of the Pentobarbital.—In approaching the mechanism of the prolongation of sleeping times of pentobarbital by the antihistamines an effort was made to see if the response to a lower than ED_{25} could be increased. Each rat of a group of fifteen averaging 108 Gm. in weight, was given a dose of 20 mg./Kg. of pentobarbital. Four out of the fifteen rats lost the righting reflex. After an interval of four days, the same group was given the same dose of pentobarbital preceded this time by 10 mg./Kg. of Ambodryl®. Again only four rats lost the righting reflex. These results suggest an action other than true potentiation; at least with this dose of pentobarbital the modification of response is not apparent.

In an effort to gain more facts which might shed light upon this altered response to pentobarbital another experiment was carried out in which fifteen rats averaging 130 Gm. in weight were each given 22.5 mg./Kg. of pentobarbital. Six of the fifteen went to sleep for three to twenty-eight minutes. Five days later the same rats were given 10 mg./Kg. of Ambodryl® followed in a half hour by 22.5 mg./Kg. of pentobarbital and in this case twelve of the

TABLE II. SIGNIFICANCE OF RESULTS

Trial	1		2		3	
	t value	*p*[a]	*t* value	*p*	*t* value	*p*
Benadryl®	2.09	0.08	1.35	0.25	2.62	0.04
Ambodryl®	2.16	0.08	2.33	0.06	3.45	0.015
Pyribenzamine®	1.50	0.19	..	..	..	..
Theophorin®	1.86	0.08	2.38	0.06	..	..
Chlor-Trimeton®	3.42	0.015	1.57	0.18	1.1	0.3

[a] *p*—probability.

fifteen rats went to sleep and slept for eleven to forty-five minutes. From an examination of these results it would seem that the dose of pentobarbital is an important factor in determining whether or not a previous dose of antihistaminic will increase the response to the pentobarbital. In raising the dose from 20 to 22.5 mg./Kg. a 50% difference resulted between the control group and the experimental group in the number of animals losing the righting reflex. This difference in the dose would suggest that a certain threshold level of circulating pentobarbital was necessary before any significant modification of response could be produced. These facts would weigh heavily in favor of the increased permeability of the brain as the cause of the increased sleeping time. Thus, in the first instance the amount of circulating unbound pentobarbital was of such a level that the concentration in the brain necessary to produce anesthesia was never attained.

The Antihistamines Cause a Mobilization of Pentobarbital in the Brain.—An experiment to ascertain whether the antihistamines increase the rate of entrance of pentobarbital into the brain was performed. Twelve rats were used and each was injected with 60 mg./Kg. of pentobarbital. Ambodryl® (10 mg./Kg.) was given intraperitoneally as each rat regained the righting reflex. Six of the twelve animals relost the righting reflex and slept for eighteen to seventy-five minutes. These results also would seem to favor the theory of increased penetration of the brain by pentobarbital.

The following experiments were undertaken to ascertain some of the chemical aspects of the phenomenon with which we are concerned. It was agreed that if it could be shown that the antihistamines interfere with the normal mechanisms of the tissues in dealing with pentobarbital, some explanation of the prolonged sleeping times might be forthcoming. Since the liver and the brain are the principal organs with which we are concerned when dealing with the barbiturates, it was decided to study the tissue concentrations of pentobarbital in rats injected with pentobarbital alone and in rats receiving both the barbiturate and the antihistamine.

The Effect of Antihistamines on the Rate of Disappearance of Pentobarbital from the Liver.—The results are shown in Fig. 2 with micrograms of pentobarbital per gram of liver plotted against time in minutes after anesthesia. A somewhat lower level of pentobarbital in the livers of the experimental animals would appear to be the logical result of a drain on any barbiturate reservoir by the brain. However, this difference was neither striking nor consistent throughout the duration of anesthesia. At the same time it must be kept in mind that the animals were assuredly not always on the same physiological plateau.

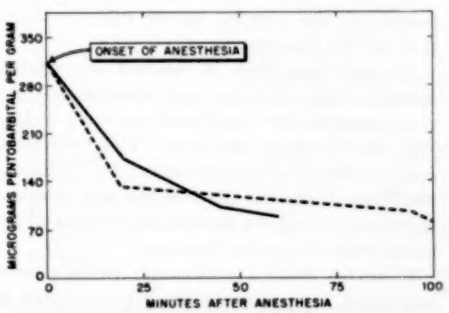

Fig. 2.—Effect of Ambodryl® on disappearance of pentobarbital from liver. ———, controls; – – – – Ambodryl®.

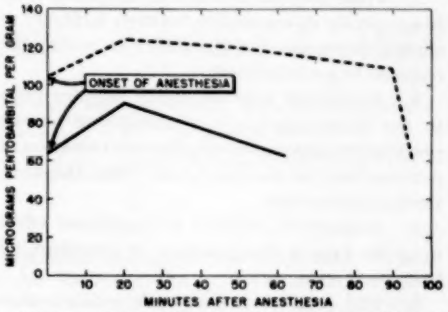

Fig. 3.—Pentobarbital levels in brain with and without Ambodryl®. ———, controls; – – – – Ambodryl®.

The Effect of the Antihistamines on the Concentration of Pentobarbital in the Brain.—In this experiment the entire brain was removed for analysis. The results are shown in Fig. 3. It would appear that the idea of increased rate of entrance of pentobarbital into the brain is borne out by these results. The concentration of pentobarbital into the brain is substantially higher at every time period from onset of anesthesia until the waking point at which time the concentrations were the same in both the control group and the experimental group. The mechanism of this enhanced entrance of the brain by pentobarbital would possibly have to be sought on an enzyme level.

Antihistamines and Enzymes.—Numerous reports have appeared indicting the antihistamines as disturbers of enzyme systems. The possibility of these actions being related to the central nervous system action of the antihistamines might well be kept in mind. Kreckowa (17) reported the antihistamines as effectively blocking the decarboxylation of pyruvate *in vitro* and also found the antihistamines increased the amount of pyruvic acid in the rabbit brain *in vivo*. Hubbard and Goldbaum (18) also found inhibition of pyruvate oxidation produced by pyribenzamine. Tickner (19) claims Benadryl®, Pyribenzamine®, Thephorin®, and Phenergan® block amine oxidase. Carlisle and Crescitelli (20) found Benadryl®, Pyribenzamine®, and Histadyl® were capable of selectively inhibiting the oxidation of *l*-glutamate while not interfering with glucose oxidation. All these facts are highly suggestive of some interference with carbohydrate metabolism of the brain and parallel some of the actions common to other hypnotics. While not a final answer to the mechanism of the depressant action of the antihistamines, they deserve further scrutiny.

SUMMARY

1. Out of a total of twenty-two trials of comparing the sleeping times of animals injected with pentobarbital alone with sleeping times of animals injected with both pentobarbital and antihistamine only on three occasions did the sleeping times of the controls exceed those of the experimentals.

2. From the results of this investigation there is apparently no connection between incidence of clinical depression and the ability to modify the response to pentobarbital.

3. Ambodryl® injected thirty minutes prior to the administration of pentobarbital in rats produces a significantly higher concentration of pentobarbital in the grain than when the anesthetic is given alone.

4. Ambodryl® produces no significant effect upon the rate of disappearance of pentobarbital from the liver in rats.

5. The results of this work indicate that there must be a critical concentration of pentobarbital in the blood before the altered response produced by the antihistamines can be shown.

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Preliminary Studies Concerning the Lyophilized Water-Soluble Extracts of *Digitalis purpurea* L. and *Digitalis lutea* L.*

By F. P. COSGROVE† and E. P. GUTH‡

A method for preparing lyophilized water-soluble extracts of *Digitalis purpurea* L. and *Digitalis lutea* L. has been described. The extracts which are instantaneously soluble in water, lend themselves readily to the extemporaneous preparations of infusions. Preliminary tests on the extracts stored approximately sixty days indicate that apparently the powders are stable and have potencies comparable with tinctures prepared from corresponding samples when equal concentrations are compared. Further studies concerning these extracts are now in progress.

DIGITALIS INFUSION has been a favorite form of digitalis medication among many physicians since its introduction to medicine in 1785 by Withering. While many investigators have found that the infusions of digitalis are prone to rapid decomposition, Weiss and Hatcher (1) found that their infusions represented more completely the potency of the digitalis leaves than did the tinctures.

Pomeroy and Heyl (2) studied the relative activity of the tinctures and infusions of equal concentrations and found infusions to be slightly less active than tinctures. This difference could be due to extraction factors, for Vartiainen and Yrjo (3) showed that one per cent infusions represented a greater percentage potency of the leaves than ten per cent infusions.

It was felt that if a stable water-soluble powder containing ingredients of the infusion could be made, some of the difficulties associated with the keeping qualities of the infusion might be reme-

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Based upon a dissertation submitted by F. P. Cosgrove to the Graduate School of The Ohio State University in partial fulfillment for the degree of Doctor of Philosophy.
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TABLE I.—MILLIGRAMS OF EXTRACT PER MILLILITER OF INFUSION

Sample	Age of Plant in Years	Method of Drying	Average mg./ml.
D. purpurea	1	Oven[a]	5.7
D. purpurea	1	Freeze-dried[b]	5.7
D. purpurea	2	Oven[a]	5.0
D. purpurea	2	Freeze-dried[b]	4.7
D. lutea	3	Oven[a]	5.8
D. lutea	3	Freeze-dried[b]	5.2

[a] Oven-dried at 50° C. for thirty-six hours.
[b] Lyophilized at 250 μ of pressure at a temperature of less than 35° C. by means of the Stokes Freeze Drying Equipment Model 2004-L.

died. In order to obtain a water-soluble extract to which the pharmacist could extemporaneously add water and compound an infusion, the freeze-drying process was employed.

EXPERIMENTAL

The source, collection, and treatment of the material used in this study has been previously described (4). Lyophilized water-soluble extracts were prepared from oven-dried and lyophilized leaves of the following plants: *Digitalis purpurea* L., first and second year plants, and *Digitalis lutea* L., third year plants.

Preparation of the Extracts.—To obtain the extracts, 1.5% aqueous infusions of the leaves were prepared by the following modified N. F. method.

Boiling distilled water, 900 ml., was poured over 15.0 Gm. of powdered digitalis, in a suitable vessel, the container was tightly covered, and the contents allowed to infuse for one hour in a warm place. The mixture was then filtered through paper and sufficient boiling distilled water passed over the residue to bring the total volume to one liter. The infusion was placed immediately into shallow dishes, stored in the freezing unit of the Stokes Freeze Drying Equipment, Model 2004-L until completely frozen and then lyophilized for twelve hours under a pressure of 250 μ and a temperature of less than 35°. The dried extract was stored in tightly stoppered bottles containing a small quantity of drying agent.

The amount of extract per milliliter of infusion is given in Table I.

TABLE II.—GLYCOSIDAL CONTENT OF THE EXTRACTS[a]

Sample	Age of Plant in Years	Method of Drying	No. of Detns.	Average Percentage Potency of Reference Standard
D. purpurea	1	Oven[b]	8	135.1
D. purpurea	1	Freeze-dried[c]	8	124.1
D. purpurea	2	Oven[b]	4	83.5
D. purpurea	2	Freeze-dried[c]	4	75.0
D. lutea	3	Oven[b]	6	126.5
D. lutea	3	Freeze-dried[c]	6	118.2

[a] Analyses were made according to the modified Knudson-Dresbach Method (5).
[b] Oven-dried at 50° C. for thirty-six hours.
[c] Lyophilized at 250 μ of pressure at a temperature of less than 35° C. by means of the Stokes Freeze Drying Equipment Model 2004-L.

Glycosidal Content of the Lyophilized Water-Soluble Extracts.—The total glycosides were determined by the modified Knudson-Dresbach method (5). A 5-ml. aliquot of an aqueous solution corresponding to a 10% infusion was used for each determination. Readings were made at 525 mμ in a 23-ml. absorption cell with the aid of a Fisher electrophotometer. Results expressed as percentage potency of reference standard are shown in Table II.

Toxicity Tests.—The extracts in physiological salt solution corresponding to 1% infusions were tested for toxicity according to the cup infusion method (6). Results appear in Table III.

DISCUSSION

All the extracts were light-brown in color and dissolved readily in water at 25°. Clear solutions could be obtained with concentrations as high as 20%. Since the extracts were slightly hygroscopic, they were stored in tightly closed bottles containing a small bag of drying agent. When equal concentrations are compared, our findings with the toxicity tests and colorimetric assays indicate that the potencies of the extracts compare favorably with the results obtained with tinctures made from corresponding samples (4).

TABLE III.—TOXICITY OF EXTRACTS ON FROGS

Sample	Age of Plant in Years	Method of Drying	No. of Frogs Used	Range of Systolic Stoppage Times[c]	Average Time for Systolic Stoppage[c]
D. purpurea	1	oven[a]	15	21–48	31.5
D. purpurea	1	Freeze[b]	15	19–45	28.9
D. purpurea	2	Oven[a]	15	41–65	50.9
D. purpurea	2	Freeze[b]	15	30–66	50.3
D. lutea	3	Oven[a]	14	24–54	42.4
D. lutea	3	Freeze[b]	14	20–60	40.4

[a] Oven-dried at 50° C. for thirty-six hours.
[b] Lyophilized at 250 μ of pressure at a temperature of less than 35° C. by means of the Stokes Freeze Drying Equipment Model 2004-L.
[c] Times are in minutes.

While the toxicity tests show no significant differences in the potency with samples made from either the oven-dried or lyophilized material, the colorimetric determinations indicate notable differences in the samples prepared from material dried by the two methods. Although it cannot be concluded, at present, whether the observations are due to differences in total glycosides, it seems possible in light of previous investigations (7, 8), that the differences may be due to variations of primary and secondary glycosides.

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Carbohydrate and Chlorophyll Content of Leaves of *Digitalis purpurea* L. and *Digitalis lutea* L. After Freeze-Drying and Oven-Drying*

By F. P. COSGROVE† and E. P. GUTH‡

The sugar and chlorophyll content of alcoholic extracts of oven-dried and lyophilized leaves of *Digitalis purpurea* and *Digitalis lutea* are reported. Freeze-drying preserves more total chlorophyll and chlorophyll "a" than oven-drying. Little difference in chlorophyll "b" was observed with material dried by either method. Oven-drying increases the amount of sugars extracted with alcohol U. S. P. from *Digitalis lutea*. Freeze-drying does not have any more effect than oven-drying on the amount of carbohydrate material extracted with alcohol U. S. P. from *Digitalis purpurea*.

IN A PREVIOUS REPORT (1) it was shown that some differences occurred in the total glycosides of extracts prepared from oven-dried and lyophilized leaves of *Digitalis purpurea* L. and *Digitalis lutea* L. This paper deals with the carbohydrate and chlorophyll content of the leaves of the same species of Digitalis.

Chambers and Nelson (2) in their work on Belladonna leaves, reported that oven-drying caused more proteolysis than freeze-drying; therefore, it was of interest in this investigation to determine if oven-drying at 50° would alter other cellular materials of the leaf such as carbohydrates and chlorophyll. It has been pointed out with leaves undergoing proteolysis that chlorophyll disappears to the same extent as the chloroplastic proteins (3). This is in accordance with Chambers and Nelson who found less chlorophyll in the oven-dried samples.

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EXPERIMENTAL

A description of the collection, treatment, and storage of the raw material has been previously reported (1). The oven-dried and lyophilized leaves of the following plants were used in this study: *Digitalis purpurea* L., first and second year plants, and *Digitalis lutea* L., second and third year plants.

Distilled water, 25 ml., was added to a 200-ml. aliquot of the total alcoholic extract and the alcohol evaporated on a steam bath. The aqueous concentrate was transferred to a 200-ml. volumetric flask and the gummy precipitate thoroughly washed into the flask with distilled water. Ten milliliters of a saturated solution of neutral lead acetate was added to precipitate the tannins, phenols, and other possible interfering substances. The mixture was shaken, made to volume with distilled water, thoroughly mixed, and filtered through dry filter paper. The first 10 ml. of the filtrate was discarded because of possible adsorption of the sugars on the dry filter. To precipitate the excess lead acetate, solid anhydrous sodium carbonate was added to the filtrate. The mixture was thoroughly mixed and filtered through dry filter paper. The first 10 ml. of filtrate was again discarded.

In order to bring about hydrolysis, a 50-ml. aliquot of the filtrate, 5 ml. of 36% hydrochloric acid, and 25 ml. of distilled water were placed into a 100-ml. volumetric flask and hydrolyzed at room temperature for fifteen hours. The mixture was neu-

tralized with solid anhydrous sodium carbonate, diluted to volume, and thoroughly mixed by shaking.

The determination for total sugar was carried out on a 50-ml. aliquot of the neutralized sample according to the Munsen and Walker method (4) using potassium permanganate to titrate the cuprous oxide. The results calculated as dextrose are given in Table I.

TABLE I.—PERCENTAGE OF SUGAR CALCULATED AS DEXTROSE

Sample	Age of Plant in Years	Method of Drying	Number of Determinations	Average Percentage
D. purpurea	1	Oven[a]	4	7.62
D. purpurea	1	Freeze-dried[b]	3	7.72
D. purpurea	2	Oven[a]	6	5.37
D. purpurea	2	Freeze-dried[b]	7	4.93
D. lutea	2	Oven[a]	6	11.24
D. lutea	2	Freeze-dried[b]	7	6.91
D. lutea	3	Oven[a]	7	11.93
D. lutea	3	Freeze-dried[b]	6	7.93

[a] Oven-dried at 50° C. for thirty-six hours.
[b] Lyophilized at 250 μ of pressure at a temperature of less than 35° C. by means of the Stokes Freeze Drying Equipment Model 2004-L.

Chlorophyll Determination.—The content of total chlorophyll, chlorophyll "A" and chlorophyll "B" was determined according to the A. O. A. C. method (5). Spectrophotometric readings were made by the use of the Beckman D. U. Spectrophotometer. The percentage of total chlorophyll, chlorophyll "A" and chlorophyll "B" was calculated on the dry weight basis of the leaves. Results appear in Table II.

DISCUSSION

Some variations of the sugar content were observed in the extracts studied. Samples prepared from the oven-dried and lyophilized leaves of *Digitalis purpurea* L. showed no significant differences in sugar content, while it was found that extracts prepared from the oven-dried leaves of *Digitalis lutea* contained considerably more sugar than those prepared from the corresponding lyophilized products. While there is a possibility that the increase in sugar is due to the extraction of more total glycosides, the colorimetric assays previously reported (1) do not indicate that such a positive relationship definitely exists. If such a great difference in total sugars was due to more glycosides being present in the alcoholic extracts of the oven-dried leaves of *Digitalis lutea*, it appears that a greater difference in the glycosidal content of these extracts should have been found with the colorimetric assays.

Since oven-drying at 50° causes proteolysis in leaves, it may be possible that this method of drying could alter the cellular or nonglycosidal sugars of the leaves of *Digitalis lutea* in such a manner that they are more readily soluble in alcohol. Further studies concerning this phase of the problem are now in progress.

An attempt was made to determine the sugar content of the aqueous extract from the marc of the alcoholic extraction, but the percentage of sugar was so small that reproducible results could not be obtained.

Since leaf material was limited, chlorophyll determinations were run in duplicates. These results indicate that lyophilization preserves more total chlorophyll than oven-drying. This is in accordance with the results obtained by Chambers and Nelson (2) with Belladonna leaves.

Although data are limited, there seems to be a striking similarity between the sugar content and the total chlorophyll. Samples having no significant differences in total sugars showed only a slight difference in total chlorophyll while those possessing a great difference in total sugars had similar differences in total chlorophyll.

The present data do not indicate any absolute relationship between the sugar content and the previously reported total glycosides as determined by the modified Knudson-Dresbach method (6).

TABLE II.—PERCENTAGE OF CHLOROPHYLL

Sample	Age of Plant in Years	Method of Drying	No. of Determinations	Percentage of Total Chlorophyll	Chlorophyll "A"	Chlorophyll "B"
D. purpurea	1	Oven[a]	2	0.64	0.34	0.18
D. purpurea	1	Freeze-dried[b]	2	0.69	0.36	0.20
D. purpurea	2	Oven[a]	2	0.50	0.22	0.17
D. purpurea	2	Freeze-dried[b]	2	0.56	0.29	0.17
D. lutea	2	Oven[a]	2	0.54	0.28	0.16
D. lutea	2	Freeze-dried[b]	2	0.69	0.38	0.19
D. lutea	3	Oven[a]	2	0.59	0.30	0.18
D. lutea	3	Freeze-dried[b]	2	0.71	0.37	0.20

[a] Oven-dried at 50° C. for thirty-six hours.
[b] Lyophilized at 250 μ of pressure at a temperature of less than 35° C. by means of the Stokes Freeze Drying Equipment Model 2004-L.

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Observations on the Metabolism of Alcohol*

By LOUIS C. SCHROETER and ARTHUR G. ZUPKO†

The effect of ethanol on the *in vitro* deposition of cholesterol and on the kidney citrate content is presented. The rate of disappearance of ethanol from the blood of rabbits is accelerated by the administration of adenosine triphosphate or fructose, but not by the administration of Chlor-Trimeton®, Pro-Banthine®, or Dormison®.

THE EFFECT of various substances on the metabolism of ethanol has been the subject of many investigations, but comparatively few studies have been done which attempt to explain the underlying mechanisms involved. The physiological effects of ethanol in man are not fully understood, especially the role of ethanol in various disease states and the manner of toxic effect production.

Lester and Greenberg (1) observed that a low incidence of arteriosclerosis occurred in alcoholics. Recently Mallov and Tepperman (2) advanced the hypothesis that ethanol produces its toxic effect by interfering with the normal operation of Krebs' cycle. These reports led us to investigate the effects of ethanol on cholesterol deposition and to endeavor to substantiate the Mallov-Tepperman hypothesis. In addition, preliminary studies were undertaken to ascertain the effect upon the rate of oxidation of ethanol by selected substances, including a sugar, a phosphate donor, an antihistamine, an anticholinergic, and a hypnotic.

EXPERIMENTAL

Methods.—The effect of ethanol on the deposition of cholesterol was studied by Bjorksten's method (3), which demonstrates that, *in vitro*, the intima of fresh hog aorta may be made to adsorb and retain cholesterol from a carbon-pigmented aqueous cholesterol suspension. The effect is produced by treatment with certain cross-linking agents of which lead acetate is one of the most efficacious. Fifteen fresh hog aortas[1] were slit open and round test sections of 7 mm. diameter were punched out of each aorta by means of a cork borer. Sections from the 15 aortas were divided into three equal groups. Group *A* was used to test the effect of pretreatment with ethanol by placing the aortas into 0.47, 0.94, 1.7, 4.5, and 30% ethanol solutions for four to five hours. Group *B* and *C* served as controls, the sections being placed into water for a period of four to five hours. After the pretreatment, all sections were removed from the solutions and washed twice with a shaking machine. Sections from groups *A* and *C* were then placed in a 3% lead acetate solution for twenty-four hours; those of group *B* were placed in water for twenty-four hours. All test sections were then immersed in the cholesterol suspension for fifteen minutes, after which they were washed twice by placing them in distilled water and shaking by machine. The sections which had been in contact with the lead acetate solution showed heavy cholesterol deposits; those which were not treated with lead acetate failed to show any deposits after washing. The presence or absence of cholesterol deposition was determined by visual examination; sections which adsorbed cholesterol appeared black as a result of the carbon inside the suspended cholesterol particles.

The method of Mallov and Tepperman (2), in which the kidney citrate content of normal and intoxicated rats was determined, was employed for the investigation of the effect of ethanol on Krebs' cycle. Adult male rats of the Anheuser-Busch strain were divided into six groups. Group *A* consisted of six rats which served as controls. Groups *B*, *C*, *D*, *E*, and *F* consisted of three rats each, all of which were fed by stomach tube. Group *B* received 7 cc. of 50% ethanol, Group *C* 7 cc. of 50% ethanol and 1 Gm. of glucose, Group *D* 7 cc. of 50% ethanol and 1 Gm. of fructose, Group *E* 1 Gm. of glucose, and Group *F* 1 Gm. of fructose. Forty-five minutes after administration of the materials under test, the rats were decapitated and samples of the kidneys were analyzed for citrate content by a modification of the method of Ettinger, *et al.* (4). This method is a simplified procedure for the oxidation and bromination of citric acid to pentabromoacetone. The pentabromoacetone is reacted with alkaline pyridine to give a colored reaction product. The modification consisted of pipetting an aliquot of the pyridine after centrifugation into a microcuvette and determining the optical density at 530 mμ in the Coleman spectrophotometer.

In order to investigate the effects of different substances on the oxidation rate of ethanol, it was necessary to prepare normal alcoholaemic curves. Fourteen rabbits were used for this purpose. Seven rabbits were fasted for twenty-four hours, ethanol being administered intravenously as a 25% solution in a dose of 0.75 Gm./Kg. Thirty minutes after the administration of the ethanol and at intervals of thirty to sixty minutes for three hours thereafter, blood samples were taken from the marginal ear vein for analysis. The determination of blood ethanol was made by a modification of the method of Johnston and Gibson (5). In this method, the ethanol is distilled into dichromate solution, sulfuric acid is introduced through the reflux tube for the oxidation. The modification employed consisted essentially of the titration of the excess dichromate with ferrous ammonium sulfate titrant and the de-

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[1] The fresh hog aortas used in this study were provided by the St. Louis Independent Packing Co., St. Louis, Mo.

termination of the end point of the reaction with the Fisher Electric Eye Titrimeter. The same dose, concentration of ethanol, and analytical technique were employed throughout this study. Four fasted rabbits were administered ethanol orally in the same dose and concentration by means of a stomach tube. Three nonfasted rabbits were administered ethanol intravenously for the purpose of comparing both the effect of feeding and of the methods of administration.

Twenty adult fasted rabbits were used to study the effects of different substances on the oxidation rate of ethanol. Five rabbits were administered ethanol intravenously followed by the intravenous administration of fructose in a dose of 1 Gm./Kg. Two rabbits were administered ethanol and fructose orally. Both solutions were administered in the same dosage as was employed for intravenous administration. Adenosine triphosphate was administered as a solution of the sodium salt simultaneously with ethanol to five rabbits. Three rabbits received 32 mg. and two rabbits received 5 and 13 mg. of sodium ATP respectively. Chlor-Trimeton® maleate was administered to four rabbits. One rabbit received 50 μg. intravenously fifteen minutes after the administration of the ethanol. A second rabbit received 50 μg. intravenously ten minutes before the administration of ethanol. The third and fourth rabbits received 300 μg. and 600 μg. intramuscularly thirty and fifteen minutes before the administration of ethanol intravenously. Pro-Banthine® was administered to two rabbits in a 5-mg. and 10-mg. intravenous dose eighteen minutes before the administration of ethanol intravenously. Dormison® was administered in a 10-mg. dose to two rabbits after they had received ethanol intravenously.

TABLE I.—EFFECT OF ETHANOL ON CHOLESTEROL DEPOSITION

	Pretreatment	Lead Acetate[a] 3% Solution	Color After Washing
1	*a* 0.47% Ethanol 4 hr.	24 hr.	Black
	b Water 4 hr.	[b]	White
	c Water 4 hr.	24 hr.	Black
2	*a* 0.94% Ethanol 5 hr.	24 hr.	Black
	b Water 5 hr.	...	White
	c Water 5 hr.	24 hr.	Black
3	*a* 1.7% Ethanol 5 hr.	24 hr.	Black
	b Water 5 hr.	...	White
	c Water 5 hr.	24 hr.	Black
4	*a* 4.5% Ethanol 4 hr.	24 hr.	Black
	b Water 4 hr.	...	White
	c Water 4 hr.	24 hr.	Black
5	*a* 30% Ethanol 5 hr.	24 hr.	Black
	b Water 5 hr.	...	White
	c Water 5 hr.	24 hr.	Black

[a] After treatment with lead acetate solution, all test sections were immersed in the cholesterol suspension for fifteen minutes.
[b] All group *b* test sections were placed in water for twenty-four hours.

RESULTS

Effect on Cholesterol Deposition.—The effect of ethanol on the deposition of cholesterol is shown in Table I. Pretreatment of sections of hog aorta by immersion into 0.47, 0.94, 1.7, 4.5, and 30% ethanol solutions did not affect the deposition of cholesterol. Bjorksten (3) originally suggested that the mechanism of the *in vitro* cholesterol deposition is due to the cross-linking of the protein in the intima, but later stated that the primary effect probably is precipitation of the protective colloid and that the cholesterol comes out with this (6). Carboxy methylcellulose serves as the protective colloid for the pigmented cholesterol suspension. In preliminary experiments with this method we found that methylcellulose solutions would not give the desired effect thus indicating that the protective colloid played an important part in the mechanism of action.

Effect on Kidney Citrate Content.—The effect of ethanol on the kidney citrate content of rats is shown in Table II. Supernormal citrate concentrations were found in the kidneys of rats which had received ethanol, ethanol and glucose, or ethanol and fructose. The values presented for both control and ethanol-intoxicated rats are somewhat lower than the corresponding values presented by Mallov and Tepperman (2).

Effect on Ethanol Metabolism.—The rates of disappearance of ethanol from the blood of 34 rabbits, 14 of which were controls, are shown in Table III. The values are presented as the mean and the range of the decline of blood ethanol expressed as mg. per 100 cc. per hour. After distribution of ethanol was complete, the elimination of ethanol from the blood approximated a linear function in all cases. With the exception of adenosine triphosphate and fructose, none of the substances investigated showed any significant acceleration of ethanol metabolism under the experimental conditions described. It should be pointed out that the results presented are only preliminary data. A more complete study of these and other agents is in progress.

DISCUSSION

Deposition of cholesterol in the arteries has been shown to play an important part in the development of arteriosclerosis. The observation of a low incidence of arteriosclerosis in alcoholics suggested an

TABLE II.—EFFECT OF ETHANOL ON KIDNEY CITRATE CONTENT

	Drugs	Number	Citrate Content per Gm. of Wet Kidney, μg.[a]		
A	Control	6	26.0	S. E.	3.7
B	Ethanol	3	55.3	S. E.	1.8
C	Ethanol and glucose	3	51.4	S. E.	5.0
D	Ethanol and fructose	3	53.4	S. E.	3.1
E	Glucose	3	28.1	S. E.	2.9
F	Fructose	3	27.4	S. E.	3.3

[a] All values presented are mean values for duplicate determinations of kidney citrate content of each animal.

TABLE III.—RATE OF DISAPPEARANCE OF ETHANOL FROM THE BLOOD OF RABBITS

Drug and Dosage	Method of Ethanol Administration	Number of Animals	Decline of Blood Ethanol[a] mg. % per Hour
Control (fasted)	I. v.	7	19 (16–23)[b]
Control (nonfasted)	I. v.	3	25 (14–33)
Control (fasted)	Oral	4	22 (16–27)
Fructose, 1 Gm./Kg. i. v.	I. v.	5	35 (28–44)
Fructose, 1 Gm./Kg. oral	Oral	2	33 (32–35)
Na ATP, 5 mg. i. v.	I. v.	1	28
Na ATP, 13 mg. i. v.	I. v.	1	30
Na ATP, 32 mg. i. v.	I. v.	3	37 (31–42)
Chlor-Trimeton®, 50 μg. i. v.	I. v.	2	24 (22–27)
Chlor-Trimeton®, 300 μg. i.m.	I. v.	1	22
Chlor-Trimeton®, 600 μg. i. m.	I. v.	1	22
Pro-Banthine®, 5 mg. i. v.	I. v.	1	20
Pro-Banthine®, 10 mg. i. v.	I. v.	1	20
Dormison®, 10 mg. i. v.	I. v.	1	22
Dormison®, 10 mg. i. m.	I. v.	1	22

[a] Calculated from samples taken one hour after administration of ethanol to allow for tissue distribution.
[b] Values not in parentheses represent mean decline of blood ethanol in all animals. Figures in parentheses represent the range.

investigation of the effects of ethanol on the *in vitro* adsorption of cholesterol by fresh hog aortas. We were unable to demonstrate that ethanol prevented *in vitro* adsorption of cholesterol by fresh hog aortas. It is apparent that further studies of the effect of ethanol on cholesterol deposition are needed. *In vivo* experimentation involving the maintenance of significant numbers of experimental and control animals on a cholesterol-ethanol diet for a long period would doubtlessly give much needed information.

Ethanol is known to be metabolized mainly in the liver in two stages; *viz.*, oxidation to acetaldehyde which is then further oxidized to acetic acid. The kidneys and other tissues play a relatively insignificant role in these steps. The acetate formed in the oxidation is either oxidized to carbon dioxide or utilized as is the acetate from other metabolites. Masoro and Abramovitch (7) reported that the kidneys surpass the liver in the rate of oxidizing ethanol to carbon dioxide. This may be a possible factor in the increased citrate levels found in the kidneys and spleen, but not the liver of ethanol-intoxicated rats. Unquestionably there are far more important factors involved.

The rate of metabolism of ethanol is not changed by many substances nor affected by many varied conditions. In our preliminary studies we found that adenosine triphosphate and fructose accelerated ethanol metabolism under the experimental conditions described. The use of adenosine triphosphate in this study was based on its involvement in carbohydrate metabolism. The nucleotide acts either as a phosphate donor or acceptor at almost every stage of this process (8). The acceleration of the oxidation rate of ethanol by fructose has been reported by several investigators (9–11). These investigators concluded that the faster disappearance of ethanol from the blood after the administration of fructose was the result of acceleration of its oxidation by pyroracemic acid (pyruvic acid). Jacobsen (12) pointed out that the effect of pyruvate on the metabolism of ethanol is controversial and that *in vitro* data on the reaction between ethanol and pyruvate and the kinetics of the process are necessary to explain the effect. Insulin and glucose have also been reported as accelerators of ethanol metabolism (13, 14); others claim these substances have no such effect (15, 16). It is apparent that considerable controversy exists as to the mechanisms involved in the effect of these substances on ethanol metabolism.

SUMMARY

1. Pretreatment of sections of hog aorta by immersion into ethanol solutions does not affect the deposition of cholesterol.

2. Supernormal citrate concentrations are found in the kidneys of rats which have received ethanol, ethanol and glucose, or ethanol and fructose.

3. The rate of disappearance of ethanol from the blood of rabbits is not affected by the administration of Chlor-Trimeton®, Pro-Banthine®, or Dormison®, but is accelerated by the administration of adenosine triphosphate or fructose.

4. Many aspects of ethanol metabolism are little understood and warrant further investigation.

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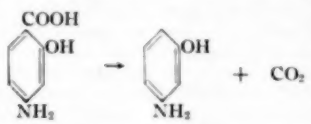
A Potentiometric Method of Assay for Sodium *p*-Aminosalicylate (Sodium PAS)*

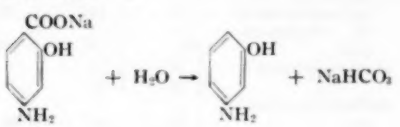
By J. RICHARD STOCKTON† and RICHARD ZUCKERMAN‡

A potentiometric assay procedure for sodium *p*-aminosalicylate (sodium PAS) and its solutions is presented. The salt is titrated with perchloric acid in propylene glycol and isopropyl alcohol. A reference standard *p*-aminosalicylate is not required. The method also simultaneously determines the extent of decomposition of the sodium salt.

THERE ARE AVAILABLE for determination of *p*-aminosalicylic acid (PAS) several methods which involve measurement of the phenolic hydroxyl and aromatic amino functions (1–6). The acid may also be directly titrated with alkali or dissolved in alcohol and determined potentiometrically with alkali. These latter methods, like the decarboxylation procedure of Oberweger, *et al.* (7), and the extraction procedure of van Steenbergen (8), possess some advantage by virtue of their dependence upon the carboxyl group, the function most readily lost during storage of PAS and its aqueous solutions:



While the sodium salt of PAS may be determined more or less precisely by some of the procedures dependent upon the phenolic and amino functions, quantitative analysis of sodium PAS and its solutions by titration with aqueous acids is not satisfactory; yet, measurement of the carboxylic function of sodium PAS is pertinent to studies of drug purity and stability. Consequently, the method of Palit (9) employing special solvent mixtures was applied to sodium PAS and proved to be suitable for determining not only the actual PAS content but also the extent of decomposition to *m*-aminophenol (and sodium bicarbonate):



The method also was found applicable to aqueous solutions of sodium PAS (such as injectable preparations) as well as to the hydrated or anhydrous salts. Indeed, in practice the inclusion of an aqueous component appeared to improve the precision of the method and was incorporated into the standard procedure.

EXPERIMENTAL

Procedure.—The sodium PAS or its aqueous solution is diluted with distilled water so that a 5-cc. aliquot contains about 2 meq. To 5 cc. of this solution in a beaker is added 10 cc. of a 1:1 (by volume) mixture of ethylene glycol and isopropyl alcohol. Into the liquid in the beaker are inserted a stirrer and the shielded electrodes from a standard *p*H meter (a Beckman Model G instrument was used in this study). While the mixture is thoroughly stirred, 0.4 to 1.0 *N* perchloric acid in ethylene glycol and isopropyl alcohol (1:1) is added in small amounts from a 2-cc. or 5-cc. microburet, the *p*H meter reading being recorded after addition of each increment. About 2–5 cc. of the acid is sufficient to complete the titration. Typical data are presented graphically in Fig. 1.

Aged solutions of sodium PAS presented data conspicuously different from fresh solutions of the salt (*cf.* Figs. 1 and 2).

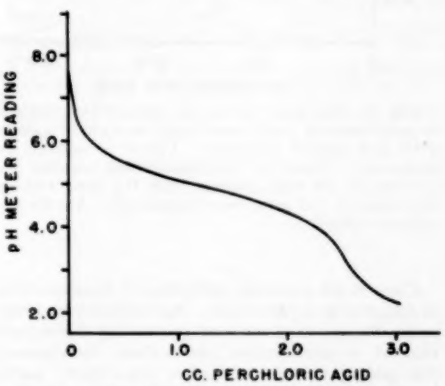


Fig. 1.—Titration curve for Sodium PAS. A 5.00-cc. aliquot of a solution containing 8.444 Gm. sodium PAS dihydrate/100 cc. was titrated with 0.795 *N* perchloric acid.

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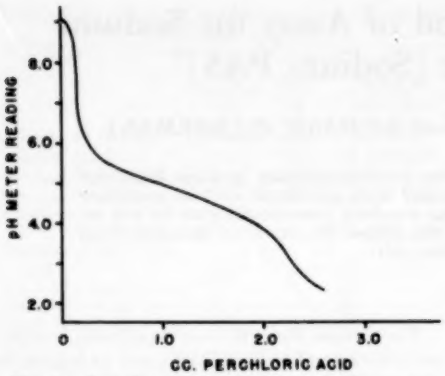

Fig. 2.—Sample titration curve for aged aqueous solution of sodium PAS.

The known components of decomposed solutions (*m*-aminophenol and sodium bicarbonate) were titrated separately and in mixtures containing sodium PAS and equimolecular quantities of sodium bicarbonate and *m*-aminophenol (Fig. 3). A titration of sodium salicylate was also included for comparison.

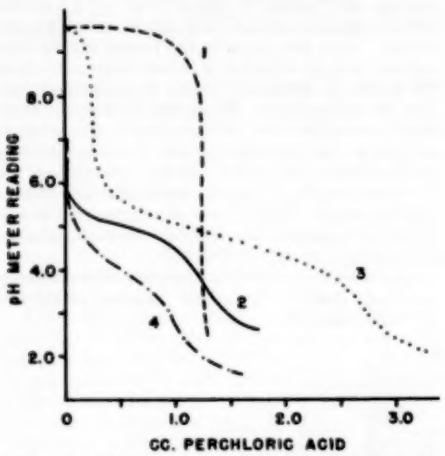

Fig. 3.—Titration curves for sodium bicarbonate, *m*-aminophenol, a simulated aged solution of sodium PAS and sodium salicylate. Curve 1—sodium bicarbonate. Curve 2—*m*-aminophenol. Curve 3—mixture of 1.8 meq. sodium PAS, 0.2 meq. sodium bicarbonate, 0.2 meq. *m*-aminophenol. Curve 4—sodium salicylate.

Color is not a reliable indication of decomposition in solutions of sodium PAS. Antioxidants may suppress discoloration without preventing decomposition to *m*-aminophenol and sodium bicarbonate. The potentiometric method is particularly useful for detecting such decomposition and for measuring the extent of decomposition.

Sodium *p*-aminosalicylate dihydrate from several manufacturers, both domestic and foreign, and many different batches, as well as anhydrous sodium *p*-aminosalicylate prepared by heating PAS with sodium ethoxide in anhydrous ethanol and by lyophilizing solutions of sodium PAS, have been titrated. The close similarity of these data rendered individual presentation in this report unnecessary.

The potentiometric method was also found applicable to determination of sodium PAS in tablets and granules and in mixtures with streptomycin and dihydrostreptomycin. The dihydrostreptomycin salt of PAS was titratable.

Calculation and Interpretation of Results.—Examination of the data in Table I and the graphs in Figs. 1, 2, and 3 reveals a single inflection point for the pure substances titrated and an additional point of inflection for the aged solution of sodium PAS and for the mixture of sodium PAS with its decomposition products. These end points, except in the case of sodium bicarbonate and other unneutralized alkali, are not sufficiently well defined to be determined accurately by inspection. However, the point of inflection may be readily obtained

TABLE I.—DETERMINATION OF END POINT

cc.	pH	Δ pH/Δ cc.	Δ² pH/Δ cc.²
2.10	4.20		
		11	
2.15			
2.20	4.09		+80
		19	
2.25			
2.30	3.90		+40
		23	
2.35			
2.40	3.67		+110
		34	
2.45			
2.50	3.33		+60
		40	
2.55			
2.60	2.93		−150
		25	
2.65			
2.70	2.68		−60
		19	
2.75			
2.80	2.49		

TABLE II.—RESULTS OF TYPICAL TITRATIONS

Product	Calcd., meq.	Found, meq.	
1. $NaPAS{\cdot}2H_2O$	2.00	2.01	1.99
2. Mixture representing 10% decomposition			
$NaPAS{\cdot}2H_2O$	1.80	1.81	1.80
$NaHCO_3$	0.20	0.184	0.192
m-Aminophenol	0.20	(0.184)	(0.192)
% decomposition	10%	9.3%	9.7%
3. *m*-Aminophenol	2.16	2.14	
	2.05	2.00	
4. Sodium bicarbonate	1.00	0.98	0.99
5. Aged solution NaPAS			
$NaHCO_3$		0.129	0.120
NaPAS (remaining)		1.53	1.50
Original NaPAS	1.63	1.66	1.62
% decomposition		7.8%	7.4%

by plotting the rate of change of pH with respect to cc. titrant added (Δ pH/Δ cc.) against cc. titrant. At the inflection point, Δ pH/Δ cc. approaches infinity.

A more satisfactory procedure is the "second derivative method" (10, 11), which involves plotting the second derivative (Δ^2 pH/Δ cc.^{2}) against cc. and determining the point at which this function becomes equal to zero. In practice this point may be calculated rather than determined graphically by simply interpolating between the last positive value and the first negative value of Δ^2 pH/Δ cc.^{2}. A sample calculation of an end point by this method may be made from the data in Table I, derived from the titration depicted in Fig. 1. The function Δ^2 pH/Δ cc.^{2} is the rate of change of Δ pH/Δ cc. with respect to cc. The value Δ^2 pH/Δ cc.^{2} = +60 through the 2.45 to 2.55 cc. range is obtained from (40–34)/(2.55–2.45). Similarly, Δ^2 pH/Δ cc.^{2} for the range 2.55 to 2.65 is equal to (25–40)/(2.65–2.55), or −150. Then, the point at which Δ^2 pH/Δ cc.^{2} equals zero may be approximated by adding to the volume of 2.50 cc., at which Δ^2 pH/Δ cc.^{2} = 60, the increment (0.1 cc. × 60/(60 + 150) = 0.03). The end point thus obtained is 2.50 + 0.03, or 2.53 cc. The same value is obtained by plotting Δ^2 pH/Δ cc.^{2} against cc.

Some typical results obtained in the titrations of several of the products mentioned are presented in Table II.

The data in Table II and the graphs of Figs. 1, 2, and 3 demonstrate that *m*-aminophenol and sodium bicarbonate, as well as sodium PAS, are titratable and that the total number of equivalents of acid, T, employed in a titration is the sum of the quantities corresponding to sodium PAS, P, *m*-aminophenol, M, and sodium bicarbonate, S:

$$T = P + M + S.$$

Since $M = S$, $T = P + 2S$. Furthermore, the amount of S is easily determined from the first sharp inflection, thereby permitting calculation of P:

$$P = T - 2S.$$

The actual calculation of (anhydrous) sodium PAS (mol. wt. 175) present in a sample aliquot may be accomplished with the formula:

$$\frac{\text{cc. acid total} - 2\,(\text{cc. acid first inflection})}{1{,}000} \times N \times 175 = \text{Gm. sodium PAS}$$

The *p*-aminosalicylic acid (PAS) content can be calculated by substituting the factor 153, the molecular weight of PAS, for the factor 175. Sodium PAS dihydrate is obtained with the factor 211. Percentage decomposition is calculated from the formula:

$$\frac{\text{cc. first inflection} \times 100}{\text{cc. total} - \text{cc. first inflection}} = \%\ \text{decomposition}$$

In the event that sodium bicarbonate and *m*-aminophenol do not occur in equimolecular proportions (as they do in the case of decomposition of sodium PAS), the method is obviously not applicable.

The precision of the method is governed by those factors usually applicable in potentiometric procedures and is diminished somewhat when the method is employed in examination of complex mixtures. The precision obtainable is indicated by the data resulting from titration of weighed samples (Table III).

TABLE III.—TITRATION OF WEIGHED SAMPLES OF SODIUM PAS DIHYDRATE WITH 1.016 N ACID

Wt. Sample, Gm.	End Point, ml.	$NaPAS{\cdot}2H_2O$ Calcd., Gm.	Difference, %
0.3303	1.537	0.3295	−0.24
0.3310	1.541	0.3304	−0.18
0.3298	1.538	0.3297	−0.03
0.3300	1.538	0.3297	−0.09

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The Influence of Experimental Hypo- and Hyperthyroidism on the Effects of Nostal®, Thiopental Sodium, and Hexobarbital Sodium in the Adult Albino Rat*

By HARALD G. O. HOLCK, IRA WM. HILLYARD,† and MARVIN H. MALONE‡

Hypothyroidism produced by either feeding suitable concentrations of propylthiouracil, or by thyroidectomy, greatly increased the average recovery time of both male and female adult albino rats to Nostal®, thiopental sodium, and hexobarbital sodium. Thyroidectomy in male rats appreciably increased the incidence of acute deaths following Nostal.® The LD_{50} of propylthiouracil injected intraperitoneally in a 5 per cent acacia suspension is 342 mg./Kg. No tolerance was built up to propylthiouracil when low concentrations were fed in the diet for two weeks. Intraperitoneal injection of propylthiouracil in acacia suspension one hour prior to Nostal® had no effect on recovery time or incidence of acute death. Delayed deaths occurred sooner. Hyperthyroidism produced by feeding effective concentrations of either thyroglobulin or thyroxin caused no consistent shortening of the average recovery time from Nostal® and did not influence the incidence of either acute or delayed death.

PREVIOUS studies indicate that hypothyroidism lowers the resistance to various drugs, such as chloroform and the ergot alkaloids (1–3). According to Monroe, such lowered resistance to chloroform in hypothyroid rats can be raised by preliminary feeding with a high carbohydrate or a high-protein diet (3). A marked increase in susceptibility to phenobarbital in the hyperthyroid animal has been noted by Raventos (4) who kept his animals in a cold environment which presumably increased thyroid functions (5). However, Sure, *et al.* (6), have observed that the detoxification of nitrogenous compounds is increased in the thyrotoxic animals, which may indicate that such an animal can more efficiently detoxify those barbiturates which are mainly broken down in the body. Because the liver is involved in this detoxification, especially of Nostal® (isopropyl-*beta*-bromallyl barbituric acid; propallylonal) with which we are mainly concerned (7–9), and because hyperthyroidism may increase the liver metabolism, a faster rate of detoxification might be anticipated in such a condition. Also, because a greater female susceptibility to Nostal® has repeatedly been observed in our rat stock, and because Benedict and Macleod (10) have reported that female rats have a 21 per cent lower basal metabolic rate than male rats, an investigation of abnormal thyroid function as it might affect the Nostal® response seemed promising. This barbiturate was chosen for the main study as it allowed recording not only recovery time from acute depression and the acute death rate, but also the number of delayed deaths and any alteration in sex variation (11, 12).

EXPERIMENTAL

General.—The rats used in this study were albinos bred originally from Wistar stock. From the time of weaning until they were to be prepared for one of the major Nostal® experiments, they were fed Purina Laboratory Chow and water *ad libitum*. The temperature of the animal quarters was maintained at approximately 26°. Whenever a drug was fed to the rats in preparation for the major acute experiments, it was thoroughly admixed with powdered Purina Laboratory Chow by an electric stirrer. In the case of thyroxin and thyroglobulin administration, involving an extremely small amount in proportion to the food, this drug was first thoroughly mixed by hand with aliquot proportions of lactose until a sufficient quantity of material had been prepared to insure proper mixing. The rats were removed from their cages and weighed just prior to administering the hypnotic in question.

Shortly before administration, Nostal® was converted to Nostal® sodium by the addition of an appropriate amount of sodium hydroxide solution, and then adding sufficient boiled, distilled water to adjust the final concentration to that employed in each test as shown in the tables. The animals were placed in individual cages and the laboratory temperature maintained at 25 ± 0.5° during the acute depression period. Following administration of a barbiturate, a rat was considered to be asleep when the righting reflex had been abolished and the animal was lying quietly on its side. Recovery was considered complete when a rat promptly retracted either hind leg from an extended position. In order

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The major portions of this article are taken from the Master's thesis of Ira Wm. Hillyard.

TABLE I.—THE INFLUENCE OF FEEDING THREE CONCENTRATIONS OF PROPYLTHIOURACIL IN THE DIET FOR TWENTY DAYS ON THE ACUTE AND DELAYED EFFECTS OF NOSTAL® UPON ADULT MALE ALBINO RATS[a]

Group	Number of Rats per Group	Mg. Propylthiouracil per Kg. of Food[b]	Mg. Propylthiouracil Eaten per Kg. per Day	Average Recovery Time Min.	*t* Factor	Number Acute Deaths	Number Delayed Deaths
A	13[c]	Food only	..	136 ± 14.8	10.4 (A *vs.* D)	1/13	2/12
B	14	50	2.7	260 ± 12.5	6.4 (A *vs.* B)	None	7/14
C	13[c]	100	5.7	264 ± 13.6	6.4 (A *vs.* C)	None	None
D	14	200	12.8	310 ± 7.8	3.4 (B *vs.* D)	3/14	1/11

[a] Sixty mg. of Nostal® per Kg. of body weight was administered intraperitoneally as a 2% solution.
[b] The propylthiouracil was thoroughly mixed with powdered Purina Laboratory Chow.
[c] These groups were reduced to 13, after one rat in each of groups A and C had died near the end of the feeding period.

to record incidences of delayed death, each group of rats was observed for five days following the final administration of Nostal®.

Experimental Hypothyroidism and the Effects of Nostal® and Thiopental upon the Adult Albino Rat.—In this phase of the study, two methods were employed to produce an experimental state of hypothyroidism, namely, (*a*) feeding of propylthiouracil in the food for a suitable length of time, and (*b*) surgical removal of the thyroid gland.

Propylthiouracil Hypothyroidism.—Two tests were carried out upon four and one-half to six and one-half month old male rats. In the first test a group of 56 rats was properly randomized into four smaller groups of 14 each, one of these groups serving as a control. The concentrations of propylthiouracil fed the three test groups were 50, 100, and 200 mg., respectively, per Kg. of food. These concentrations were suggested by the work of Astwood, Bissell, and Hughes (13), and Vanderlaan and Bissell (14), to insure complete thyroid inhibition. The various groups were fed the plain and medicated food for a period of twenty days, and then injected with 60 mg. of Nostal® per Kg. of body weight. This dosage in past experiments (9, 11, 15) had induced an average depression of approximately two hours' duration in normal male rats, usually without any incidence of acute deaths, and with delayed deaths ranging from 40 to 60%. During the twenty-day period the control animals gained 3.9% in body weight, and the three propylthiouracil groups lost 3.0, 3.8, and 4.7%, respectively. This cannot be explained as being due to a distaste for the medicated food, for the average daily food consumption was 64 Gm./Kg. of rat in the control group and 55, 57, and 63 Gm. per Kg. for the propylthiouracil-fed groups, which probably became less active as thyroid hormone function diminished. That such decrease occurred is indicated by the fact that feeding the same concentrations of propylthiouracil in the subsequent thiopental tests reduced the oxygen consumption from 22 to 29% in subgroups of three rats after nine to ten days of feeding this drug, as determined by a modified Haldane closed system method (16–18).

The results of the first test with Nostal® are summarized in Table I and show that the feeding of propylthiouracil in these concentrations increased significantly the recovery time ($p < 0.001$); the highest concentration was the most effective. No significant change was seen in regard to the incidence of acute death; the number of delayed deaths, always quite variable with Nostal® in small-sized groups, was too irregular to permit any definite conclusions.

In the second experiment 40 mg./Kg. of thiopental sodium was substituted for the 60 mg./Kg. of Nostal®. Twenty-four five to six month old male rats were divided into three groups of eight each. The two test groups were fed propylthiouracil concentrations of 50 and 200 mg./Kg. of food, respectively, for twelve days, whereas the control group was given powdered food only.

The results of this experiment are summarized in Table II. Although the average depression time was markedly increased by propylthiouracil feeding, the *t*-factors were smaller than in the case of Nostal® because we studied fewer rats and probably also because the medicated food was fed for only twelve days rather than for twenty days as in the case of Nostal.® However, because in each case p is less than 0.02 the difference is again significant. It should be noted here that the diminution of oxygen consumption when tested upon several rats from each group was fully as great with the low as with the high concentration of propylthiouracil after nine days of feeding. The premedication did not influence the incidence of acute death. In both the twelve- and the twenty-day feeding tests the rats gained in weight during the first week, but had be-

TABLE II.—THE INFLUENCE OF FEEDING PROPYLTHIOURACIL IN THE DIET FOR TWELVE DAYS ON THE ACUTE EFFECTS OF THIOPENTAL SODIUM IN ADULT MALE ALBINO RATS[a]

Group	Number of Rats per Group	Mg. Propylthiouracil per Kg. of Food	Mg. Propylthiouracil Eaten/Kg. of Rat/Day	Average Recovery Time, Min.	*t* Factor
A	8	Food only	..	137 ± 28.0	.....
B	8	50	3.1	254 ± 33.0	2.70 (A *vs.* B)
C	8[b]	200	13.4	235 ± 21.8	2.77 (A *vs.* C)

[a] The propylthiouracil was thoroughly mixed with powdered Purina Laboratory Chow; 40 mg. of thiopental sodium per Kg. of rat was injected intraperitoneally as a 2% solution.
[b] One rat died acutely in this group; thus, the average recovery time is based on seven rats.

gun to lose weight when weighed on the 12th and 14th day, respectively, whereas the control animals grew progressively heavier.

Control Experiments upon Propylthiouracil.—To make certain that the prolonged recovery time from Nostal® depression after the feeding of propylthiouracil was not due to synergism between the two drugs, the following experiment was undertaken. After preliminary toxicity studies, 39 male rats were divided into one group of three, and three groups of 12 animals each. These groups were medicated as shown in Table III.

In the group receiving propylthiouracil alone, characteristic tremors appeared, but no acute or delayed deaths occurred. In the acacia control group, the usual Nostal® depression was observed; no animals died acutely, but 58% died during the next four days. In the case of the rats premedicated with 100 and 200 mg. of propylthiouracil, 92% and 83%, respectively, died within twenty-four hours, while only 16% of the acacia group died this soon. The average recovery times for the two propylthiouracil-treated groups did not differ significantly from that in the group receiving acacia only.

1:20,000 and 1:10,000 propylthiouracil, respectively. At the end of fourteen days, the average weight of the rats in the control group had increased from 183 to 186 Gm., whereas in the group on the lower concentration of propylthiouracil it had decreased from 189 to 187, and that on the higher concentration from 191 to 185 Gm. Each rat then was given a dose of 364 mg./Kg. of propylthiouracil, administered as a 4% suspension in 5% acacia solution, intraperitoneally. The fatalities were as follows for the three groups: 4/12, 8/11, and 4/12. Considering the usual variations in mortality with small groups of animals, apparently the two-week feeding of low concentrations of propylthiouracil did not influence the resistance to a high dose of this drug.

Propylthiouracil Effects Upon the Adult Rat Response to Hexobarbital.—The Nostal® tests, to be discussed in the next section, indicated that the depression time of thyroidectomized male rats approached that of the normal females. Consequently, an experiment was conducted to ascertain if a similar effect would be observed with hexobarbital sodium (Sodium Evipal®) to which a much

TABLE III.—COMPARISON OF RECOVERY TIME AND INCIDENCE OF ACUTE AND DELAYED DEATH FOLLOWING INTRAPERITONEAL INJECTION OF PROPYLTHIOURACIL ONE HOUR PRIOR TO ADMINISTRATION OF NOSTAL® TO MALE RATS[a]

Group	Medication	Number of Animals Used	Number Acute Deaths	Total Number Delayed Deaths in		Average Recovery Time, Min.[d]
				1 Day	4 Days	
1	200 mg./Kg. Propylthiouracil alone[b]	3	0	0	0	...
2	5% Acacia prior to Nostal®[c]	12	0	2	7	163 (12)
3	100 mg./Kg. Propylthiouracil prior to Nostal®[b]	12	1	10	11	155 (11)
4	200 mg./Kg. Propylthiouracil prior to Nostal®[b]	12	2	8	9	173 (10)

[a] The male rats were 5 to 6 months old; 70 mg. of Nostal® per Kg. of body weight was injected subcutaneously as a 3.5% solution.
[b] The propylthiouracil was administered as a 2% suspension in 5% acacia solution in groups 1 and 4, and as a 1% suspension in acacia in group 3.
[c] Three cc per rat of 5% solution of acacia were injected intraperitoneally.
[d] Figures in parentheses represent the number of rats upon which the average is based.

Propylthiouracil Lethal Dose Determination.—To determine the approximate LD_{50} of propylthiouracil, administered as a 4% suspension in 5% acacia solution, and to ascertain if any sex-variation existed, 35 five to eight month old female and 40 five to six month old male rats were randomized into eight groups. Starting with 286 mg./Kg. of propylthiouracil, successive doses were increased by 4.7%. Except in case of delayed death in one male rat, all fatalities occurred within 6.8 hours; this male showed mottled lungs and liver on post-mortem examination. There was no apparent sex variation and the LD_{50} at 19/20 confidence limits, as determined by the method of Litchfield and Wilcoxon (19), was 342 (327 to 358) mg./Kg. The unusual steepness of the curve is indicated by the value of 1.108 (1.046 to 1.173) for *S*.

Determination of Possible Development of Tolerance to Propylthiouracil.—To determine if feeding of low concentrations of propylthiouracil in the diet would raise the resistance to a single high dose of propylthiouracil, 35 female rats, five to nine months old, were randomized into three approximately equal groups. These groups were fed powdered Purina Laboratory Chow and such chow containing

greater sex-difference exists, using propylthiouracil to reduce thyroid function. Twenty males and 23 females, four to five months old, were randomized according to age and weight into a test and a control group for each sex. The test groups were fed 200 mg. of propylthiouracil per Kg. of food, whereas the control groups were given food only. With this concentration of propylthiouracil, a marked increase in recovery time from Nostal® previously had been noted, accompanied by a fall in metabolic rate. The results as tabulated in Table IV show that although the recovery time of the propylthiouracil-fed groups is significantly increased for each sex—similar to the results with Nostal®—there still is a significant difference between the recovery time of the hypothyroid males and the normal females ($p < 0.001$); also that the sex-variation in case of the premedicated rats could be due to chance is excluded.

Thyroidectomy Hypothyroidism.—Groups of about seventy rats of each sex were randomized according to age, into two groups for each sex. They were subjected to thyroidectomy when the rats were approximately two and one-half months old. The removal was done under light ether anes-

TABLE IV.—THE INFLUENCE OF FEEDING PROPYLTHIOURACIL IN THE DIET FOR TWELVE DAYS ON THE SEX DIFFERENCE IN RESPONSE OF ADULT RATS TO HEXOBARBITAL SODIUM[a]

Group	Sex	Number of Rats per Group	Mg. Propylthiouracil per Kg. of Food	Average Recovery Time, Min.	t Factor
A	Male	9	Food only	24 ± 1.7	13.0 (A *vs.* C)
B	Male	11	200	53 ± 3.7	7.1 (A *vs.* B)
C	Female	11	Food only	81 ± 4.5	4.8 (B *vs.* C)
D	Female	12	200	127 ± 5.4	11.3 (B *vs.* D)
					6.8 (C *vs.* D)

[a] Hexobarbital sodium, 80 mg./Kg. of body weight was administered as a 4% solution.

thesia, care being exercised to leave the parathyroid glands and the recurrent laryngeal nerves undisturbed. Nostal® was administered in the usual manner two and one-half to three and one-half months after the operation. Preliminary tests were conducted to ascertain the approximate dose range and the remainder of the operated rats were used in the following two experiments.

The Effect of Thyroidectomy on the Male Rat Response to Nostal®.—Forty-eight thyroidectomized and 50 control male rats were randomized into five groups of nearly equal size. To determine the effect of thyroidectomy upon the LD_{50} of Nostal®, a dosage range of 69.0 to 131.5 mg./Kg. with three intermediate doses was chosen as shown in Table V; the logarithmic increase was 0.0700.

The LD_{50} for the control males is estimated to be 108.3 ± 6.5 mg./Kg. as determined by the maximum likelihood method outlined by Finney (20). In the thyroidectomized rats it was only 87.7 ± 5.2 mg./Kg. The LD_{50} for the controls was 1.25 times that for the thyroidectomized rats, with 99% fiducial limits of 1.01 and 1.68 for this ratio; thus the difference is significant at the 1% level of probability.[1] The recovery time from depression was markedly longer in the operated group on the lowest dose as compared with that of the control group; on this dose only did a sufficient number survive for statistical evaluation. However, the incidence of delayed death was not influenced by the thyroid removal. The total number of acute deaths was 18/50 in the control, against 28/48 in the operated rat groups. The combined number of acute and delayed deaths was 38/50 *versus* 39/48.

The Effect of Thyroidectomy on the Female Rat Response to Nostal.®—To compare the effects of Nostal® in the two sexes 50 normal and 49 thyroidectomized female rats were used. The dosage range was considerably lower, namely 50.0 to 95.3 mg./Kg., than in case of the males to compensate for the expected lower female resistance. Unfortunately, as seen in Table V, we overcompensated for this in that no rats died acutely on the lower three of the five doses employed for the female rats. The average female recovery time in both normal and operated rats was distinctly longer than in case of the males when all received a dose of 69.0 mg./Kg. With acute deaths occurring in only two groups, no LD_{50} can be estimated for the females. The total number of acute deaths was 11/50 in the normal *versus* 8/49 in the thyroidectomized females and the combined acute and delayed deaths were 34/50 *versus* 28/49. The lengthening of the average recovery time by thyroidectomy is much less marked than in case of the males; in fact, in view of the large standard errors, there is a significant difference only with

[1] Statistical analysis by courtesy of Professor Charles O. Gardner.

TABLE V.—THE INFLUENCE OF THYROIDECTOMY ON RECOVERY TIME AND ON THE INCIDENCE OF ACUTE AND DELAYED DEATH FOLLOWING NOSTAL® ADMINISTRATION IN THE ADULT RAT[a]

Females				Mg. Nostal® per Kg. of Body Weight	Males		
Number Acute Deaths	Number Delayed Deaths	Average Recovery Time, Min.	Group		Number Acute Deaths	Number Delayed Deaths	Average Recovery Time, Min.
0/10	3/10	183 ± 16.1	A[b]	50.0	...	...	.....
0/10	0/10	224 ± 29.5	B				
0/10	7/10	256 ± 19.2	A	58.7	...	...	.....
0/9	2/9	313 ± 38.0	B				
0/10	7/10	371 ± 32.1	A	69.0	0/10	7/10	136 ± 8.3
0/10	7/10	459 ± 24.8	B		1/10	4/9	310 ± 34.0
4/10	3/6	.....	A	81.1	2/10	3/8	.....
5/10	4/5	.....	B		4/10	3/6	.....
7/10	3/3	.....	A	95.3	3/10	4/7	.....
3/10	6/7	.....	B		8/10	1/2	.....
...	.....	.....	A	119.9	6/10	3/4	.....
...	.....	.....	B		8/9	1/1	.....
...	.....	.....	A	131.5	7/10	3/3	.....
...	.....	.....	B		7/9	2/2	.....
11/50 + 23/39 = 34/50			A	Totals	18/50 + 20/32 = 38/50		
8/49 + 19/41 = 28/49			B	Totals	28/48 + 11/20 = 39/48		

[a] Nostal®, as the sodium salt, was administered intraperitoneally as a 4% solution.
[b] Group A, control rats. Group B, thyroidectomized rats.

TABLE VI.—THE INFLUENCE OF FEEDING THYROGLOBULIN IN THE DIET FOR FIVE DAYS ON THE ACUTE AND DELAYED EFFECTS OF NOSTAL® IN THE ADULT MALE ALBINO RAT[a]

Group	Mg. Thyroglobulin per Kg. of Food	Mg. Nostal® per Kg. of Body Weight	Number Acute Deaths	Number Delayed Deaths	Average Recovery Time, Min.
A	Food only	60.0	0/18	9/18	155 ± 15.0 (18)[c]
B	11.0[b]	60.0	0/17	4/17	141 ± 8.5(17)
C	Food only	90.0	9/18	9/9	295 ± 48.0(9)
D	11.0[b]	90.0	8/18	10/10	241 ± 11.9(10)

[a] Nostal® as the sodium salt, was administered intraperitoneally as 2% and 3% solutions, respectively.
[b] The average amount of thyroglobulin consumed per Kg. of rat per day was 0.74 mg.
[c] Figures in parentheses represent the number of rats upon which the average is based.

the dose of 69.0 mg./Kg. ($t = 2.2$; $p = 0.05$), and even this is problematic. There is no clear-cut influence of the operation upon either the incidence of acute or delayed deaths; only with the two lowest doses is there any diminution in the number of delayed deaths, and in view of considerable variation of these, this difference is probably not significant. With only the 69.0 mg./Kg. dose is it possible to compare the effect of thyroidectomy with that of the average male recovery time, which is normally shorter than in the female. After the operation, the male recovery time approaches that of the normal female to such an extent that there no longer is any significant difference (p = about 0.2). However, the data are too few from which to draw any general conclusions as to the effect of thyroidectomy upon the sex-difference to Nostal®. The difference in recovery time between the operated rats of both sexes on this dose is significant ($p < 0.01$).

Effects of Experimental Hyperthyroidism upon the Responses of the Rat to Nostal®.—To produce hyperthyroidism, thyroglobulin or thyroxin was fed in sufficient concentrations (in powdered Purina Laboratory Chow) for five days to raise the oxygen consumption. The concentrations were such that the rats would be expected to absorb approximately the amounts of these substances which are found to be effective when injected subcutaneously.

Thyroglobulin Hyperthyroidism and the Male Rat Response to Nostal®.—Seventy-five male rats, three to four months old, were randomized according to age and weight into four nearly equal groups. Two of these groups were fed a concentration of 11 mg. of thyroglobulin per Kg. of food for five days prior to and for five days following Nostal® administration, the latter being the period during which delayed deaths occur. Dr. Kroc informed us that this concentration raises the oxygen consumption of young adult rats approximately 15% in five days and 40% in fourteen days (17). To study the duration of acute depression and the incidence of acute deaths, and also the incidence of delayed deaths, we chose one dose of Nostal® which would not kill any rat acutely, and another well within the acute death range.

Our results shown in Table VI indicate that the feeding of thyroglobulin significantly altered neither the average recovery time, nor the incidence of acute or delayed deaths.

Thyroxin Hyperthyroidism and the Female Rat Response to Nostal®.—In our second set of tests upon hyperthyroidism, the more active thyroxin (21, 22) was administered in two different concentrations, adapted from the subcutaneous doses of 0.06 to 0.61 mg./Kg. used by Barker, *et al.* (23), to raise the oxygen consumption of his rats about 60%. One hundred and eight three to six month old female rats were randomized into six groups. Two groups served as controls, two were fed 3.75 mg. of thyroxin per Kg. of food, and the remaining two groups were fed 7.5 mg. of thyroxin per Kg. of food. In view of a moderate sex-variation to Nostal®, the two doses were reduced sufficiently to obtain approximately the same degree of depression and number of fatalities that were obtained in the previous experiment upon male rats.

The results, tabulated in Table VII, show that only with the higher concentration of thyroxin in the food, causing a marked rise in metabolic rate, was the recovery time appreciably shorter than for the control animals—both receiving the smaller dose of Nostal® (p = about 0.04), which did not cause any acute deaths. The thyroxin feeding had no influence on either the incidence of acute or delayed deaths.

Thyroxin Hyperthyroidism and the Male Rat Response to Nostal®.—The higher concentration of thyroxin was fed as before to 28 male rats, three to six months old, 29 similar rats serving as controls. Thyroxin per Kg. of rat weight consumed per day was 0.66 mg. during the five days preceding Nostal® medication; during four days of feeding the oxygen consumption rose 24% in five of the thyroxin-fed rats, *versus* 3% in five controls, whereas, in groups of six litter mates the blood cholesterol level in five days was +2.2% in the controls and −27.5% in the thyroxin-fed rats, as determined by the Lieberman-Burchard colorimetric reaction as modified for blood determination by Bloor (24). When 60 mg. of Nostal® per Kg. of rat was administered intraperitoneally, one rat died acutely in each group, 16/28 of the controls and 20/27 of the thyroxin-fed rats died during the next few days; the average recovery time in minutes was 164 ± 7.5 for the controls and 151 ± 6.6 for the pretreated rats. Thus, there was no significant effect of hyperthyroidism upon the male rat response to Nostal®.

DISCUSSION

The most striking influence of experimental hypothyroidism is the lengthening of the recovery time of the male rat to Nostal®, thiopental, or hexobarbital. A smaller increase is shown proportionally at three dosage levels of Nostal® in female rats made hypothyroid by feeding propylthiouracil. Bilateral thyroidectomy of adult males increases the incidence of acute death as seen in the study using five doses in the fatal range. However, no such effect could be clearly seen in the two fatal-range doses in the case of female rats. Thyroidectomy reduced somewhat the incidence of delayed death following Nos-

TABLE VII.—THE INFLUENCE OF FEEDING TWO CONCENTRATIONS OF DL-THYROXIN IN THE DIET FOR FIVE DAYS ON THE ACUTE AND DELAYED EFFECTS OF NOSTAL® IN THE ADULT FEMALE ALBINO RAT[a]

Group	Mg. Thyroxin per Kg. of Food	Dose of Nostal, mg./Kg.	Number Acute Deaths	Number Delayed Deaths	Average Recovery Time, Min.
A	Food only	50	0/18	15/18	243 ± 14 (18)[d]
B[b]	3.75	..	0/18	17/18	225 ± 19 (18)
C[b]	7.5	..	0/18	16/18	203 ± 14 (18)
D	Food only	75	6/18	12/12	491 ± 27 (12)
E[c]	3.75	..	6/18	12/12	524 ± 34 (12)
F[c]	7.5	..	6/18	11/12	466 ± 25 (12)

[a] Nostal®, as the sodium salt, was given intraperitoneally as a 2.5% solution. The thyroxin feeding was continued for five days after Nostal® medication.
[b] Thyroxin per Kg. of rat weight consumed per day was 0.26 mg. for groups B and E, and 0.54 mg. for groups C and F during the five days prior to Nostal® medication.
[c] Oxygen consumption averaged +7% as determined upon four rats of one low-concentration, and +52.3% as determined upon four rats of one high-concentration group.
[d] Figures in parentheses represent number of rats upon which the average is based.

tal® given in a dosage not causing acute death. However, in view of the considerable variability in the number of delayed deaths in the control groups, it would take much larger groups of rats to make certain that this is a general rule.

Our results with thiopental are not in accord with the report by Ederstrom (25) who did not find any increase in the recovery time of rats with thiopental after giving them 0.1% thiouracil in the drinking water, even though the animals showed a 23% reduction in oxygen consumption as a result of this preliminary medication. Although we administered the more potent propylthiouracil (13, 14) in proportionally lower concentrations in the food, not in the drinking water, and injected somewhat larger doses of thiopental intraperitoneally rather than subcutaneously, it would seem that his results and ours should be in the same direction because the reduction of oxygen consumption was approximately the same with both types of premedication. Since we got practically the same increase in recovery time with two different propylthiouracil concentrations in the food, we have no explanation for the difference in Ederstrom's and our results.

The barbiturates used in this study are to a considerable extent detoxified in the liver (7). One may, therefore, assume that the capacity of the liver for detoxification of these substances is diminished by inhibition of thyroid hormone synthesis (13, 21, 26), as hypothyroidism presumably reduces the metabolism of the liver. The puzzling finding is that while the prolongation of recovery time is consistent, any effect upon the incidence of acute or delayed death is apparently much less certain. The effect upon recovery time from Nostal® is not due to an immediate effect of propylthiouracil administration because this drug when given in considerable dosage an hour before Nostal® does not increase the average recovery time. However, it apparently markedly accelerates the time of delayed death after Nostal® administration so that many more rats are dead within one day. The reason for this is unknown.

The results of one of our experiments with Nostal® indicate that whereas a considerably higher male resistance exists in normal rats, thyroidectomy of the male rat makes the recovery time approach that of the normal female (Table VI). However, suppression of thyroid hormone formation by feeding propylthiouracil failed to cause the recovery time of the treated male rats after hexobarbital to approximate that of the normal females. With hexobarbital there is a very wide sex difference in normal rats. Furthermore, although the recovery time from hexobarbital was lengthened in both sexes by feeding propylthiouracil, a significant sex difference still exists in the rats with depressed thyroid function.

In each of the three experiments, when rats were fed effective concentrations of thyroglobulin or thyroxin and the Nostal® doses did not cause acute deaths or only an occasional acute death, the recovery time was slightly shorter in the hormone-treated rats as compared with the control rats. The incidence of acute or delayed death was not materially influenced by the feeding of either thyroid principle, although the metabolic rate was definitely increased by such medication, and the blood cholesterol level, determined in one experiment, was lowered. Thus, increasing the metabolic rate by these feedings does not seem appreciably to influence the response of the rat to Nostal.®

SUMMARY

1. Hypothyroidism, produced by feeding suitable concentrations of propylthiouracil in the diet for ten or twenty days, greatly increased the recovery time of male rats after administration of hypnotic doses of Nostal®, thiopental, and hexobarbital. In the female the effect was much less striking. Counting acute deaths only, the LD_{50} of Nostal® in the normal male rat was 109 mg./Kg.; in the thyroidectomized, only 88. The effect upon the incidence of delayed death was uncertain.

2. The LD_{50} of propylthiouracil, administered intraperitoneally as a suspension in 5% acacia solution, was 342 mg./Kg.; the resistance of the two sexes was similar.

3. No tolerance was developed to propylthiouracil by feeding this drug in the diet in low concentrations over a period of two weeks.

4. Intraperitoneal administration of 100 or 200 mg. of propylthiouracil per Kg. of rat weight one hour prior to subcutaneous administration of 70 mg. of Nostal® per Kg. did not significantly influence the acute recovery time, nor the inci-

dence of acute death. However, delayed deaths occurred much sooner with this premedication.

5. Hyperthyroidism, produced by feeding either thyroglobulin or thyroxin in the food for five days had no effect on the incidence of either acute or delayed death after Nostal® in either sex although oxygen consumption was considerably increased and the blood cholesterol levels distinctly lowered. However, there was a tendency for a slightly shorter recovery time, but in only one of the three determinations did the difference approach a significant one.

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The Effect of Selected Chemicals on the Alkaloidal Yield of *Datura tatula* Linné*

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A study was made on the effect of naphthoxyacetic acid, zinc insulin, and diethylstilbestrol on the alkaloidal yield of *Datura tatula* Linné under field conditions. In each case Tween® 20 was added to the test and control solutions. Diethylstilbestrol did not appreciably affect the percentage of alkaloid in the test plants, while plants treated with naphthoxyacetic acid and with zinc insulin did show some effect in regards to alkaloid content. Data concerning the dry weight of the plants indicated that Tween® 20, when applied to the soil surrounding the plant, may be a factor in causing an increase in dry weight, but further study is necessary before conclusions can be drawn.

THE CURRENT INTEREST in the relationship of plant and animal hormones and their effect on plants has raised the question whether these hormones have a pharmaceutical application in respect to effects on medicinal plants and their constituents.

The relationship of plant and animal hormones may be summarized in the following manner. Some plant constituents reportedly can cause physiological activity in animals which is similar to that caused by several animal hormones (1–4). In turn, some animal hormones have an effect on plants (5–16). While plants and animals produce their own respective hormones, some plant hormones have been found in animal excretions (17, 18), and animal hormones have been found in plant extracts (2, 4).

Thus, it appears that in many cases there is a relationship between animal and plant hormones and that the metabolic activity of a plant may be altered by treatment with various animal and plant hormones. If there is a change in the metabolic activity of a plant, then a shift in the amount and perhaps even the type of constituents within the cell could be expected. With this in mind, the following study was undertaken to determine the effect of certain selected chemicals on the alkaloidal yield of *Datura tatula* Linné.

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EXPERIMENTAL

A preliminary experiment was conducted to ascertain which of several selected chemicals showed the most promise of affecting the alkaloid content, to discover the most effective method of application of the chemical to the plants, and to find the proper concentration of chemical to apply to the plant.

These chemicals were either plant or animal hormones or hormone-like substances. They were as follows: γ-indole-3-*n*-butyric acid, β-naphthoxyacetic acid, α-naphthaleneacetic acid, zinc insulin, thyroxin, diethylstilbestrol, anterior pituitary (acid extract and alkaline extract).[1] Experiments were conducted using the three following concentrations: 0.1, 0.001, and 0.0001%. Two different methods of applying the chemicals were employed: application to the soil and spraying the foliage.

From the results of the preliminary experiment, naphthoxyacetic acid, diethylstilbestrol, and zinc insulin were chosen for further experimentation with reference to change in alkaloidal content of *Datura tatula* Linné.

An experimental plot of 343 plants was marked off at random to provide seven different treatments sets: zinc insulin, diethylstilbestrol, and naphthoxyacetic acid test plants; a control for each test set; and one set of plants which received no solution, thus serving as a control group.

The plants received treatment three times: (*a*) when the plants were at the age of seven, (*b*) eight, and (*c*) nine weeks from the seedling stage.

The naphthoxyacetic acid test plants were treated by applying to the soil around each test plant 200 ml. of aqueous naphthoxyacetic acid test solution containing 0.5 mg. of naphthoxyacetic acid and 2 ml. of Tween® 20.[2]

The zinc insulin test solution, consisting of 0.01% zinc insulin, 1.0% Tween® 20 and 0.2% ethyl alcohol in a Clark and Lubs buffered solution of *p*H 3 (19) was sprayed on the foliage of the test plants.

The diethylstilbestrol test solution, consisting of an aqueous solution of 0.01% diethylstilbestrol, 1.0% Tween® 20 and 0.1% ethyl alcohol was applied in the same manner as the zinc insulin test solution.

Random samples were selected from the experimental plot when the plants were at the age of eight, ten, and thirteen weeks from the seedling stage. At thirteen weeks the plants were well into the fruiting stage. The third harvest for the zinc insulin test and control plants was at sixteen weeks rather than thirteen.

At each harvest five entire plants of each group were gathered, dried, and separated into leaf, root, and stem portions and then pooled. At the last harvest the dried fruit was also separated and retained.

The dry weight of the plant parts was noted as an indication of comparative growth. These results are shown in Table I.

After the plant parts were dried and weighed, they were powdered in a Wiley mill and mixed well to insure homogeneous samples.

TABLE I.—DRY WEIGHT OF ABOVE-GROUND PORTION OF PLANTS

	Weight in Grams		
	1st Harvest	2nd Harvest	3rd Harvest
No treatment	1,069	2,227	3,346
Naphthoxyacetic acid control	1,197	2,506	4,731
Naphthoxyacetic acid test plants	994	2,149	3,876
Diethylstilbestrol control	1,057	2,201	3,054
Diethylstilbestrol test plants	743	1,959	3,505
Zinc insulin control	1,078	1,056	2,650
Zinc insulin test plants	1,220	1,346	2,681

The official assay, as given in *The National Formulary*, IX (20), was used for the alkaloidal assay of the samples. Three assay determinations were made of each sample. The assay values, which are expressed on a moisture-free basis, are given in Table II. The results of the statistical analysis of the alkaloidal assay data are given in Table III. The student *t* value was used as a measure of significance. The *t* value was calculated by pooling the variance of all the assay determinations of the plants analyzed 140 degrees of freedom, and testing the hypothesis that the means of any two samples being compared are the same.[3] A *t* value greater than 2.61 was considered significant at a 1% level. The formulas used are given at the end of Table III.

Table IV lists the amount of alkaloid, in grams, contained in the above-ground portions of each set of plants, representing equal sized plots. If a plant has a high percentage of alkaloid but is small in size, it may contain less total alkaloid than a larger plant with a smaller percentage of alkaloid. Therefore, for practical purposes, total yield is of greater importance than percentage of alkaloid.

DISCUSSION

Naphthoxyacetic Acid.—It is evident from the data in Table I that the plants which received the naphthoxyacetic acid control solution, an aqueous 1.0% solution of Tween® 20, were greater in dry weight than any of the other sets of plants. Furthermore, because of this greater dry weight, it can be observed in Table IV that in equal-sized plots of ground the naphthoxyacetic acid control plants would contain more total alkaloid than any of the other sets of plants. The best inference is that the Tween® 20 in the control solution which was applied to the soil surrounding the plants caused this increase in dry weight. Mitchell and Linder (21) in 1950 reported that Tween® 20 increased the rate of absorption and translocation of radioactive 2,4-D by bean plants. Thus the Tween® 20 solution added to the soil possibly could have caused greater absorption from the soil of materials which are utilized by the plant. A further study is necessary before it can be concluded whether Tween® 20 can bring about an increase in dry weight of a plant.

[1] The authors are indebted to the following companies for materials furnished for this experiment: Winthrop-Stearns, Inc., for diethylstilbestrol; Eli Lilly and Co., for zinc insulin; Organon, Inc., for thyroxin; Armour and Co., for anterior pituitary.

[2] Polyoxyethylene sorbitan monolaurate made by Atlas Powder Co., Wilmington, Del.

[3] The statistical procedure was suggested and approved by The Statistics Laboratory, The Ohio State University.

TABLE II.—RESULTS OF ALKALOIDAL ASSAY OF THE SAMPLE (EXPRESSED IN PER CENT—MOISTURE FREE BASIS)

	Leaf Sample, Assay Number				Stem Sample, Assay Number				Root Sample, Assay Number				Fruit Sample, Assay Number			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
First Harvest																
Nontreated	.575	.595	.580	.583	.389	.379	.355	.374	.460	.491	.493	.481				
Diethylstilbestrol control	.510	.534	.517	.520	.433	.447	.443	.441	.532	.504	.489	.508				
Diethylstilbestrol test	.574	.548	.578	.567	.495	.478	.481	.485	.556	.528	.530	.538				
Naphthoxyacetic acid control	.568	.543	.560	.557	.302	.318	.319	.313	.450	.464	.469	.461				
Naphthoxyacetic acid test	.566	.580	.534	.560	.424	.432	.428	.428	.454	.448	.484	.462				
Zinc insulin control	.605	.635	.644	.628	.468	.476	.458	.467	.527	.479	.512	.506				
Zinc insulin test	.460	.497	.499	.485	.244	.271	.265	.260	.477	.468	.464	.470				
Second Harvest																
Nontreated	.530	.566	.531	.542	.233	.234	.226	.231	.475	.454	.495	.475				
Diethylstilbestrol control	.593	.591	.582	.589	.236	.225	.231	.231	.488	.443	.483	.471				
Diethylstilbestrol test	.515	.517	.508	.513	.364	.354	.399	.372	.364	.354	.399	.372				
Naphthoxyacetic acid control	.730	.698	.697	.708	.267	.255	.255	.259	.397	.386	.405	.396				
Naphthoxyacetic acid test	.623	.626	.635	.628	.236	.223	.211	.223	.429	.421	.373	.408				
Zinc insulin control	.793	.760	.750	.768	.374	.380	.371	.375	.320	.377	.369	.355				
Zinc insulin test	.632	.649	.641	.641	.266	.314	.306	.295	.423	.419	.444	.429				
Third Harvest																
Nontreated	.771	.683	.712	.722	.225	.209	.207	.214	.318	.282	.289	.296	.852	.838	.832	.841
Diethylstilbestrol control	.656	.650	.659	.655	.180	.185	.189	.185	.300	.274	.288	.287	.784	.739	.755	.759
Diethylstilbestrol test	.706	.718	.733	.719	.112	.119	.124	.118	.269	.287	.295	.284	.765	.799	.764	.776
Naphthoxyacetic acid control	.770	.773	.781	.775	.204	.198	.190	.197	.305	.301	.301	.302	.773	.791	.911	.825
Naphthoxyacetic acid test	.865	.836	.846	.849	.272	.273	.266	.270	.342	.324	.356	.341	.697	.770	.830	.766
Zinc insulin control	.959	.955	.959	.958	.112	.118	.177	.136	.311	.339	.282	.311	.979	1.001	.990	.990
Zinc insulin test	1.113	1.119	1.086	1.106	.157	.129	.138	.141	339	.339	.346	.341	.933	.871	.974	.926

TABLE III.—RESULTS OF STATISTICAL ANALYSIS OF THE ALKALOIDAL ASSAY DATA

Compared Samples	Leaf Sample		Stem Sample		Root Sample		Fruit Sample	
	Diff.[a]	*t* value	Diff.	*t* value	Diff.	*t* value	Diff.	*t* value
First Harvest								
Nontreated, diethylstilbestrol control	0.063	2.526	0.067	2.690[b]	0.027	1.084		
Nontreated, diethylstilbestrol test	0.016	0.642	0.111	4.457[b]	0.057	2.289		
Nontreated, naphthoxyacetic acid control	0.026	1.044	0.061	2.449	0.020	0.803		
Nontreated, naphthoxyacetic acid test	0.023	0.923	0.054	2.168	0.019	0.763		
Nontreated, zinc insulin control	0.045	1.807	0.093	3.734[b]	0.025	1.004		
Nontreated, zinc insulin test	0.098	3.935[b]	0.114	4.578[b]	0.011	0.441		
Diethylstilbestrol control, diethylstilbestrol test	0.047	1.887	0.044	1.767	0.030	1.204		
Naphthoxyacetic acid control, naphthoxyacetic acid test	0.003	0.120	0.115	4.618[b]	0.001	0.040		
Zinc insulin control, zinc insulin test	0.143	5.742[b]	0.207	8.313[b]	0.036	1.445		
Second Harvest								
Nontreated, diethylstilbestrol control	0.047	1.887	0.000	0.000	0.004	0.160		
Nontreated, diethylstilbestrol test	0.028	1.124	0.141	5.663[b]	0.103	4.136[b]		
Nontreated, naphthoxyacetic acid control	0.166	6.666	0.028	1.124	0.079	3.132[b]		
Nontreated, naphthoxyacetic acid test	0.086	3.453[b]	0.008	0.321	0.067	2.690[b]		
Nontreated, zinc insulin control	0.226	9.076[b]	0.144	5.783[b]	0.120	4.819[b]		
Nontreated, zinc insulin test	0.099	3.975[b]	0.064	2.570	0.046	1.847		
Diethylstilbestrol control, diethylstilbestrol test	0.076	3.052[b]	0.141	5.663[b]	0.099	3.975[b]		
Naphthoxyacetic acid control, naphthoxyacetic acid test	0.080	3.212[b]	0.036	1.445	0.012	0.481		
Zinc insulin control, zinc insulin test	0.127	5.100[b]	0.080	3.212[b]	0.074	2.971		
Third Harvest								
Nontreated, diethylstilbestrol control	0.067	2.690	0.029	1.164	0.009	0.361	0.082	3.293[b]
Nontreated, diethylstilbestrol test	0.003	0.120	0.096	3.855[b]	0.012	0.481	0.065	2.610
Nontreated, naphthoxyacetic acid control	0.053	2.128	0.017	0.682	0.006	0.240	0.016	0.642
Nontreated, naphthoxyacetic acid test	0.127	5.100[b]	0.056	2.249	0.045	1.807	0.075	3.012[b]
Nontreated, zinc insulin control	0.236	9.477[b]	0.078	3.132[b]	0.015	0.602	0.151	6.064[b]
Nontreated, zinc insulin test	0.384	15.421[b]	0.073	2.931[b]	0.045	1.807	0.085	3.413[b]
Diethylstilbestrol, control, diethylstilbestrol test	0.064	2.570	0.067	2.690[b]	0.003	0.120	0.017	0.682
Naphthoxyacetic acid control, naphthoxyacetic acid test	0.074	2.971[b]	0.073	2.931[b]	0.039	1.566	0.059	2.369
Zinc insulin control inc insulin test	0.148	5.943[b]	0.005	0.200	0.030	1.204	0.064	2.570

[a] Difference of the two means.

[b] Significant *t* value, where $t = (\bar{x} - \bar{y})/\sqrt{s^2/_n\, V^2}$; $\bar{x}$ and $\bar{y}$ are any two means being compared and V^2 is the pooled variance. V^2 = Sum of the squares of the 210 assay determinations − 3 × the sum of the square of the 70 means.

Due to the report of Mitchell and Linder (21) Tween® 20 was included in the naphthoxyacetic acid test solution with the thought in mind that it might aid in the rate of absorption and translocation of the naphthoxyacetic acid as it did for the 2,4-D in Mitchell and Linder's experiment. While the plants which received the naphthoxyacetic acid test solution did not show the same increase in dry weight as the naphthoxyacetic acid control plants, they did show an increase in percentage of alkaloid. From the study of Tables II and III, it can be observed that the leaves of the naphthoxyacetic acid test plants at the third harvest contained a significantly greater percentage of alkaloid than the

leaves of the control and the nontreated plants. The stems of the naphthoxyacetic acid test plants at the second and third harvest contained a significantly greater percentage of alkaloid than the stems of the control plants.

TABLE IV.—AMOUNT OF ALKALOID PRESENT IN THE ABOVE-GROUND PORTIONS OF THE HARVESTED PLANTS

Sample	First Harvest Total Alkaloid, Gm.	Second Harvest Total Alkaloid, Gm.	Third Harvest Total Alkaloid, Gm.
Nontreated	5.29	8.61	13.93
Diethylstilbestrol control	5.14	8.83	12.91
Diethylstilbestrol test	3.95	7.04	13.21
Naphthoxyacetic acid control	5.39	11.85	21.35
Naphthoxyacetic acid test	4.52	9.06	19.03
Zinc insulin control	6.04	6.22	10.67
Zinc insulin test	4.73	6.54	12.71

Zinc Insulin.—It can be seen from Table I that the plants sprayed with the zinc insulin test solution, consisting of 0.01% zinc insulin, 1.0% Tween® 20, and 0.2% ethyl alcohol in a Clark and Lubs buffered solution of *p*H 3, and the plants sprayed with the control solution, consisting of the same solution as the test solution except that it did not contain the zinc insulin, had in general considerably less dry weight than the nontreated plants. The zinc insulin test plants were slightly greater in dry weight than the control plants. In addition to the small dry weight these two groups of plants were also slower in maturing and for this reason the third harvest was not until the plants were 16 weeks old. At this age they seemed to be in the same stage of fruiting as the other sets of plants were at 13 weeks of age.

The zinc insulin test and control solutions were buffered at a *p*H of 3, in order to dissolve the zinc insulin crystals. It has been shown that culture solutions having a *p*H below 4 or above 9 are not suitable for good plant growth because of injury to plant roots (22). Although the solutions for this experiment were sprayed on the foliage and were not in contact with the roots, it may be that the acid solution was injurious enough to inhibit the proper growth of the plants.

From the study of Table II it can be observed that at first the percentage of alkaloid was less in the leaf samples of the control. However, at the third harvest, the percentage of alkaloid in the leaf samples of the zinc insulin test plants was significantly greater than the percentage of alkaloid in the leaf samples of the zinc insulin control and nontreated plants. The zinc insulin control leaf samples of the second and third harvest and stem samples of the first and second harvest were greater in percentage of alkaloid than the corresponding samples of nontreated plants. The fruit samples of both the zinc insulin test and control plants were significantly greater in percentage of alkaloid than the fruit sample of the nontreated plants. In terms of total alkaloid, expressed in grams (Table IV), the zinc insulin control and test plant samples contained the least amount of alkaloid of all the samples. This was due to the fact that they were smaller plants.

The action of zinc insulin on the plant cannot at this time be explained. Further experimentation is being carried out to ascertain whether the effect of the zinc insulin was due to the zinc, the insulin, or a combination of both.

Diethylstilbestrol.—It has been reported (10) that diethylstilbestrol increased plant growth, particularly enhancing leaf growth. The results of this experiment do not confirm that report. The dry weight of the diethylstilbestrol-treated plants, as given in Table I, is not significantly different from the dry weight of the control plants and nontreated plants.

Study of Tables II and III will show that although the diethylstilbestrol test and control plants varied from a greater to a smaller percentage of alkaloid than the nontreated plants, no definite pattern emerged.

It was interesting to note that the data in Table II in this experiment agree with previously reported (23) data for *Datura alba*. The order of the plant organs in decreasing magnitude of percentage of alkaloid was fruit, leaves, roots, and stems. The leaves increased in percentage of alkaloid with each harvest, whereas the stems and roots decreased with each harvest.

SUMMARY AND CONCLUSIONS

1. A study was carried out concerning the effect of naphthoxyacetic acid, zinc insulin, and diethylstilbestrol on the alkaloidal yield of *Datura tatula* Linné. The study was carried out under field conditions with the naphthoxyacetic acid being applied to the soil, whereas the zinc insulin and diethylstilbestrol were sprayed on the foliage. In each case the test solution and control solution contained 1.0 per cent Tween® 20. This served as a spreading agent in the case of the solutions sprayed on the foliage. In the case of the naphthoxyacetic acid it was thought the Tween® 20 might aid in the rate of absorption and translocation of the naphthoxyacetic acid.

2. The leaves and stems of the plants treated with the naphthoxyacetic acid and Tween® 20 contained a significantly higher percentage of alkaloid at the third harvest (the fruiting stage) than the leaves and stems of the plants which received only the 1.0 per cent solution of Tween® 20. (Tables II and III.)

3. The dry weight of the plants that received the naphthoxyacetic acid control solution, an aqueous 1.0 per cent Tween® 20 solution, was greater than the dry weight of the plants that received the naphthoxyacetic acid test solution, which contained both the Tween® 20 and naphthoxyacetic acid, and the dry weight of the nontreated plants (Table I). Evidence pointed to

Tween® 20 as a possible factor in this increase of dry weight. Further study is to be carried out along this line.

4. The leaves of the plants treated with zinc insulin and Tween® 20 in Clark and Lubs buffer solution, *p*H 3, contained a significantly higher percentage of alkaloid at the third harvest (the fruiting stage) than the leaves of the plants which received only the 1.0 per cent solution of Tween® 20 in the *p*H 3 solution and the nontreated plants.

5. Diethylstilbestrol did not appreciably affect the percentage of alkaloid in the test plants nor did the treated plants show any great difference from the control plants in dry weight.

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A Comparison of a Spectrophotometric and the Silver Salt Titration Methods for the Determination of Theophylline in Various Pharmaceuticals*

By J. P. COMER and W. W. HILTY

A method is described for the spectrophotometric determination of theophylline in powders, injections, capsules, tablets, and suppositories. The method is based on the absorption of theophylline in 0.1 *N* hydrochloric acid solution at 270 mμ. The results obtained by the spectrophotometric method maintain or improve the precision and accuracy of the results obtained by the silver salt titration method performed on the same pharmaceuticals.

THE ASSAY of theophylline by the silver salt titration method was developed by Stevens and Wilson in 1937 (1) and later became the official method in the Second Supplement of the U. S. P. XI (2). This method has been the basis for the determination of theophylline in filled capsules of aminophylline-ephedrine-amobarbital (3) and solutions of aminophylline-vinbarbital (4). The latter reference evaluated an inherent error in the U. S. P. XIV assay method caused by the solubility of silver theophyllinate in ammonium hydroxide solution.

A study of the U. S. P. XIV method and a modification omitting the use of ammonia has been reported and certain recommendations made for improving the present assay (5). The potentiometric titration of theophylline in aminophylline-phenobarbital mixtures has been reported (6).

The silver salt titration procedure presents certain disadvantages when used for colored or complex mixtures which interfere with the precipitation of the silver theophyllinate, slow the filtration time, or obscure the end point of the titration. The purpose of the study presented here was to develop a method for the determination of theophylline in various pharmaceuticals which would be less time-consuming and more versatile than the U. S. P. XIV method and

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which would maintain or improve the accuracy and precision obtainable by the U. S. P. XIV method.

An ultraviolet spectrophotometric method for the assay of theophylline in blood and tissues has been reported by Schack and Waxler (7). These authors used the absorption at 277 mμ in 0.1 *N* sodium hydroxide solution as a measure of the theophylline content. The method presented here is based upon the absorption of theophylline in 0.1 *N* hydrochloric acid solution at 270 mμ. The results obtained by this method are compared with those obtained by the silver salt titration procedure.

EXPERIMENTAL

General.—For both methods, five samples were weighed from the same lot of material prepared in the manner described in the separate experiments. The absorbancies of the theophylline solutions were measured in a Beckman Quartz Model DU spectrophotometer in 1-cm. matched silica cells using 0.1 *N* hydrochloric acid as the reference solution.

The silver salt titration procedures given in the U. S. P. XIV method were used when applicable. When deviations from the U. S. P. XIV methods were necessary, they are noted in the description of the separate experiments.

Anhydrous Theophylline.—A one-tenth gram sample of anhydrous theophylline was dissolved in 200 cc. hot water in a 1,000-cc. volumetric flask. The solution was cooled and diluted to 1,000 cc. with distilled water. A 10-cc. aliquot of this solution was transferred to a 100-cc. volumetric flask, 1 cc. concentrated hydrochloric acid added, and the solution diluted to 100 cc. with distilled water. The absorbancy of this solution was measured at 270 mμ. The absorption spectrum between 200 mμ and 300 mμ of a solution similarly prepared is shown in Fig. 1.

Duplicate loss on drying determinations of the theophylline sample showed 0.18% and 0.12% loss. The average loss indicated a purity of 99.85%. The average of the five absorbancies found by the method above was 0.544. This value divided by 99.85 gives a theoretical absorbancy of 0.545 for 10 μg. of theophylline in 0.1 *N* hydrochloric acid. This value was confirmed by recrystallizing anhydrous theophylline in methanol, drying the crystals for two hours in a vacuum at 105° and determining the absorbancy by the method described above. The absorbancies of solutions containing 6, 7, 8, 9, 10, and 11 μg. of theophylline in 0.1 *N* hydrochloric acid were found to be 0.328, 0.382, 0.436, 0.489, 0.545 and 0.600, respectively. Thus solutions of theophylline in 0.1 *N* hydrochloric acid obey the absorption laws for the concentrations used in this study. For the calculations reported here a standard absorbancy for 10 μg. of anhydrous theophylline in 0.1 *N* hydrochloric acid of 0.545 was used.

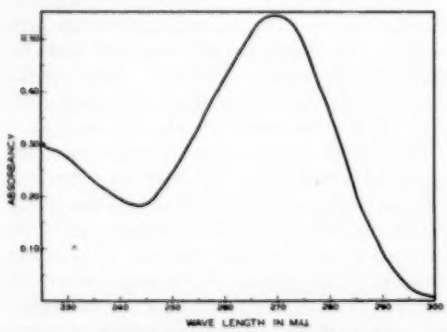

Fig. 1.—Absorption spectrum of anhydrous theophylline, 10 μg./cc., in 0.1 *N* hydrochloric acid *vs.* 0.1 *N* hydrochloric acid in 1-cm. matched silica cells.

Aminophylline Powder.—A sample of 0.125 Gm. of aminophylline was dissolved in sufficient distilled water to make 1,000 cc. of solution. A 10-cc. aliquot of this solution and 1 cc. concentrated hydrochloric acid were diluted to 100 cc. and the absorbancy of the resulting solution measured at 270 mμ.

Theophylline Monoethanolamine Powder.—A sample of 0.120 Gm. of theophylline monoethanolamine was dissolved and diluted and the absorbancy of the resulting solution measured in the manner described for aminophylline.

TABLE I.—COMPARATIVE ASSAY RESULTS FOR POWDERS

	Powder		
	Theophylline, %	Aminophylline, %	Theophylline Monoethanolamine, %
Average % theophylline, 5 determinations by method			
Silver salt	98.8	81.5	73.7
Spectrophotometric	99.8	81.0	73.3
Standard deviation of 5 determinations by method			
Silver salt	±0.4	±0.2	±0.2
Spectrophotometric	±0.2	±0.2	±0.1
Theophylline, theoretical	99.8[a]	78.9[b]	74.7[b]

[a] 0.2% loss on drying.
[b] Calculated from theoretical molecular ratios.

Aminophylline Injection (0.25 Gm. in 10 cc.).—Ampuls of aminophylline injection, purchased from a retail store, were broken and the solutions pooled. A 10-cc. aliquot of the pooled solution was diluted to 1,000 cc. with distilled water. Then a 10-cc. portion of the latter dilution plus 2.5 cc. concentrated hydrochloric acid were diluted to 250 cc. with water. The absorbancy of the resulting solution was measured at 270 mμ.

Aminophylline Tablets (0.1 Gm.).—The weight of 100 aminophylline tablets, purchased from a retail store, was determined, and the average tablet weight calculated. The tablets were powdered and a sample weight equivalent to 5 tablets transferred to a 1,000-cc. volumetric flask. The powder was shaken with water for two minutes and then diluted to 1,000 cc. The mixture was allowed to settle, then filtered and the first 50 cc. of filtrate discarded. A 10-cc.

aliquot of the filtrate and 5 cc. concentrated hydrochloric acid were diluted to 500 cc. with water. The absorbancy of the resulting solution was measured at 270 mμ.

A sample weight of the powdered mixture equivalent to 5 tablets was mixed with 5 cc. of ammonia test solution, diluted to 100 cc., and filtered. A 20-cc. aliquot of the colored filtrate was used for the U. S. P. XIV method. The colored solution made the detection of the end point difficult.

TABLE II.—COMPARATIVE ASSAY RESULTS FOR AMINOPHYLLINE INJECTION (0.25 GM.) AND AMINOPHYLLINE TABLETS (0.1 GM.)

	Injection Method		Tablets Method	
	Silver Salt	Spectrophotometric	Silver Salt	Spectrophotometric
Av. theophylline, 5 determinations	1.90%	1.92%	83.8 mg.	80.6 mg.
Standard deviation of 5 determinations	±0.01%	±0.01%	±0.6 mg.	±0.1 mg.
Theophylline theory	1.97%		78.9 mg. per tablet	
Sample size	10 cc.	10 cc.	10 tablets	5 tablets

Filled Capsules Theophylline Monoethanolamine (0.0972 Gm.).—The weight of the contents from 20 filled capsules was determined and the average weight per capsule calculated. A sample weight equivalent to five capsules was shaken in a 1,000-cc. volumetric flask with 200 cc. distilled water for two minutes, then diluted to 1,000 cc. with water and filtered. A 10-cc. aliquot of the filtrate and 5 cc. concentrated hydrochloric acid were diluted to 500 cc. with water, and the absorbancy of the resulting solution read at 270 mμ.

A sample weight equivalent to the content of 10 capsules was mixed with 5 cc. ammonia T. S. and diluted to 100 cc. This mixture was filtered and a 25-cc. aliquot used for the U. S. P. XIV method.

Filled Capsules Theophylline Monoethanolamine (0.0972 Gm.) and Amobarbital (0.0324 Gm.).—The weight of the contents from 20 filled capsules was determined and the average weight per capsule calculated. The sample weight and procedure were the same as those used for the spectrophotometric method described for filled capsules theophylline monoethanolamine.

A sample weight equivalent to the contents of 10 capsules was mixed with 5 cc. ammonia T. S. and diluted to 100 cc. This mixture was filtered, and a 50-cc. aliquot used for the U. S. P. XIV method.

Suppositories Theophylline Monoethanolamine (0.5 Gm.).—The weight of 20 suppositories was determined, and the average weight per suppository calculated. The suppositories were melted, mixed thoroughly, and allowed to solidify. A sample weight of the solid equivalent to one suppository was transferred to a separatory funnel and 50 cc. ether added. The mixture was extracted with three 200-cc. portions of water. The aqueous extracts were collected in a 1,000-cc. volumetric flask and diluted to 1,000 cc. with water. A 10-cc. aliquot of the latter and 5 cc. concentrated hydrochloric acid were diluted to 500 cc. with water and the absorbancy of the resulting solution measured at 270 mμ.

The U. S. P. XIV method for suppositories of aminophylline was performed on a sample weight of the solid suppository mix equivalent to two suppositories.

DISCUSSION

Anhydrous theophylline powder is the only pharmaceutical product assayed during this investigation which contained a known amount of theophylline. The assay results of the theophylline powder show that the spectrophotometric method gave the theoretical amount of theophylline while the silver salt method average result was 1% low. This is believed to be a significant difference and probably can be explained by the solubility of silver theophyllinate in ammonium hydroxide solutions.

The actual amount of theophylline in the products containing theophylline monoethanolamine and aminophylline was not known. The theoretical amounts of theophylline shown in the tables were calculated from the theoretical molecular ratios of

TABLE III.—COMPARATIVE ASSAY RESULTS FOR FILLED CAPSULES

	Theophylline Monoethanolamine (0.0972 Gm.) Method		Theophylline Monoethanolamine (0.0972 Gm.) and Amobarbital (0.0324 Gm.) Method	
	Silver Salt	Spectrophotometric	Silver Salt	Spectrophotometric
Average theophylline, 5 determinations	72.8 mg.	73.8 mg.	76.8 mg.	76.7 mg
Standard deviation, 5 determinations	±0.5 mg.	±0.1 mg.	±0.3 mg.	±0.3 mg.
Theophylline theory	72.6 mg. per capsule		72.6 mg. per capsule	
Sample size	10 capsules	5 capsules	10 capsules	5 capsules

Timed Disintegrating Tablets of Theophylline Monoethanolamine (0.1944 Gm.).—The weight of 50 tablets was determined and the average weight per tablet calculated. The tablets were finely ground, and a sample weight equivalent to five tablets was shaken with 200 cc. of water for five minutes and then diluted to 1,000 cc. and filtered. A 5-cc. aliquot of the filtrate and 5 cc. concentrated hydrochloric acid were diluted to 500 cc. with water and the absorbancy of the resulting solution measured at 270 mμ.

TABLE IV.—COMPARATIVE ASSAY RESULTS FOR TIMED DISINTEGRATING TABLETS (0.194 Gm.) AND SUPPOSITORIES (0.5 Gm.) OF THEOPHYLLINE MONOETHANOLAMINE

	Tablets		Suppositories	
	Method		Method	
	Silver Salt	Spectrophotometric	Silver Salt	Spectrophotometric
Average theophylline, 5 determinations	140.2 mg.	145.8 mg.	413.3 mg.	382.0 mg.
Standard deviation of 5 determinations	±1.5 mg.	±1.0 mg.	±7.7 mg.	±1.1 mg.
Theophylline theory	145.2 mg. per tablet		373.4 mg. per suppository	
Sample size	5 tablets	5 tablets	2 suppositories	1 suppository

theophylline and the monoethanolamine or ethylenediamine. These values are the indications but not the proofs of the amounts of theophylline actually present.

The average values for the aminophylline and theophylline monoethanolamine powders found by both methods agree within the limits of a routine control procedure. The differences in precision of the two methods for the assay of the powders are not significant.

The methods agree in both precision and accuracy for the aminophylline injections. The excipients of normal uncoated tablets and of capsule powders do not interfere with the spectrophotometric method for the dilutions used in this study. The spectrophotometric method is slightly more precise than the silver salt method for aminophylline tablets.

The spectrophotometric average is about one per cent higher than the silver salt average for capsules of theophylline monoethanolamine. Again the precision found by the spectrophotometric method appears to be slightly better.

Both methods agree in both precision and average for the filled capsule of amobarbital and theophylline monoethanolamine. This shows that the amobarbital causes no more interference in the spectrophotometric method than it does in the silver salt method. Amobarbital was found to give practically no absorption at 270 mμ in the dilutions of 0.1 *N* hydrochloric acid used for this assay.

The dye used in the coating of the tablets of theophylline monoethanolamine made the detection of the end point difficult by the silver salt method. The excipients of this tablet do not interfere with the spectrophotometric procedure described. The average of the results of the silver salt method was about four per cent higher than that of the spectrophotometric method. The precision was essentially the same for both methods.

The silver salt method is not applicable for the suppositories of theophylline monoethanolamine since the individual results were erratic and the averages high. The spectrophotometric method described is sufficiently accurate and precise for the assay of this suppository.

No attempt was made to collect data concerning the time required to complete the five determinations of the various products by both methods. From experience the generalization can be made that the spectrophotometric method requires approximately one-fourth the time necessary to complete the silver salt titration procedure.

SUMMARY

1. A method is described for the spectrophotometric determination of theophylline in powders, injections, capsules, tablets, and suppositories.

2. The results obtained by the spectrophotometric and the silver salt titration method for the determination of theophylline in the above pharmaceuticals are compared.

3. The results indicate that the spectrophotometric method could be expected to maintain or improve the accuracy and precision obtainable by the silver salt titration method when used for the assay of theophylline in the products mentioned in this study.

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The Essential Oil of *Amphiachyris dracunculoides* (DC) Nutt.*

By A. C. GLASSER,† L. E. HARRIS, B. V. CHRISTENSEN, and F. W. BOPE

Steam distillation of the flowering tops of *Amphiachyris dracunculoides* (DC) Nutt. yielded 1.6 per cent of an essential oil. Physical and chemical properties of the oil are given. The oil has been fractionally distilled and the components investigated.

Amphiachyris dracunculoides (DC) Nutt., Compositae is one of two species of *Amphiachyris* native to the south central portion of the United States. The plant has been called *Brachyris dracunculoides* DC, *Brachyris ramosissima* Hook., and *Amphiapappus dracunculoides* Torr. and Grey. Nuttal gave the plant its present name in 1841 (1). Local names for the plant are Broom Weed and August Flowers (2). The plant grows in dry soil and is widely distributed throughout the Southwest. The plant blooms in September and October.

The use of *Amphiachyris dracunculoides* as a home remedy for coughs and colds was established in the southwestern part of the United States many years ago. An infusion of the flowering tops is used to make a syrup.

The plants used in this study were collected near Mangum, Okla. The flowering tops, consisting of the upper stem, leaves, and inflorescence were taken as the sample, and were air dried.

During the course of a phytochemical investigation of the plant an essential oil was isolated. The initial report of the properties and the characterization of the oil of *Amphiachyris dracunculoides* follows.

EXPERIMENTAL

Isolation of the Oil.—The air-dried flowering tops were ground in a Wiley mill using an 8 mesh screen. A total of 8.1 Kg. of this material was steam distilled in a Lloyd extractor. The distillate was collected in 3,000-cc. separatory funnels and the water was drained off as the oil separated. The oil was dried over anhydrous sodium sulfate, and after filtering, approximately 142 cc. of a pale yellow, limpid liquid of not unpleasant odor was obtained for a yield of 1.6% of the dried material.

Physical and Chemical Properties.—Various physical and chemical properties of the oil were determined as follows: d_4^{25} 0.9135, n_D^{25} 1.4784 α_D^{25} −15°36′, acid number 1.06, ester number 26.1, ester number after acetylization 64.8, alcohol content as borneol 10.6%, carbonyl content 19% by the bisulfite method.

Separation of the Oil.—The oil was separated chromatographically on silicic acid by the method of Kirchner (3) into a hydrocarbon and an oxygenated fraction. Four hundred grams of silicic acid was packed as a slurry in petroleum ether into a column 4.5 x 54 cm., constricted at the bottom, and carrying a cotton plug under a layer of sand. Reduced pressure of 65 mm. was applied to the bottom of the column, and when the column failed to shrink further, 90 cc. of the oil was added and drawn onto the column. Petroleum ether was then added as the eluting agent. The appearance and presence of terpenes in the eluate was determined by means of the fluorescein-bromine test and the chromatostrips of Kirchner (4). Terpenes appeared after 300 cc. of petroleum ether had been added, and after 2,300 cc. had been added a negative test was obtained. The eluting agent was then changed to ethyl acetate, and 800 cc. was needed to elute the oxygenated compounds. The solvents were removed at 0° by distillation at the reduced pressures of 10 mm. for ethyl acetate and 25 mm. for petroleum ether. In this manner 65 cc. of colorless, limpid, pleasant-smelling hydrocarbons was obtained, and 23 cc. of a yellow liquid with the odor of the original oil was obtained as the oxygenated fraction. This amounted to 97.7% recovery of the initial sample.

Carbon and hydrogen determinations (C, 88.35; H, 11.58) showed the absence of oxygenated compounds in the hydrocarbon fraction.

Fractional Distillation of the Hydrocarbons.—Fifty-five cubic centimeters of the hydrocarbons were fractionally distilled through a column 1.2 x 30 cm., packed with one-eighth inch, single-turn glass helices. The column was provided with an external heating coil and carried a still head of the Whitmore-Lux design. Fractionation was started at atmospheric pressure, but after the fourth fraction the pressure was reduced to 24 mm. Thirty fractions were collected by intermittent take-off, and the physical properties are summarized in Fig. 1.

Three major separations are evident from Fig. 1, and on the basis of these separations three samples were prepared, consisting of fractions 7–15, 18–23, and 25–30. Each combined sample was distilled twice from sodium.

The presence of three major constituents was confirmed through chromatographic examination of the crude hydrocarbon fraction by means of the silicic acid chromatostrips with hexane as a developer. Upon examination of the strips by means of the fluorescein-bromine test, three substances were apparent as yellow spots on a pink background. Each of the purified, combined samples was also examined by this method for homogeneity, and was found to contain only one compound giving a positive fluorescein-bromine test.

* Received August 21, 1953, from the College of Pharmacy, The Ohio State University, Columbus, Ohio.

Presented to the Scientific Section, A. Ph. A., Salt Lake City meeting, August, 1953.

Based upon a dissertation submitted by A. C. Glasser to the Graduate School of The Ohio State University in partial fulfillment for the degree Doctor of Philosophy.

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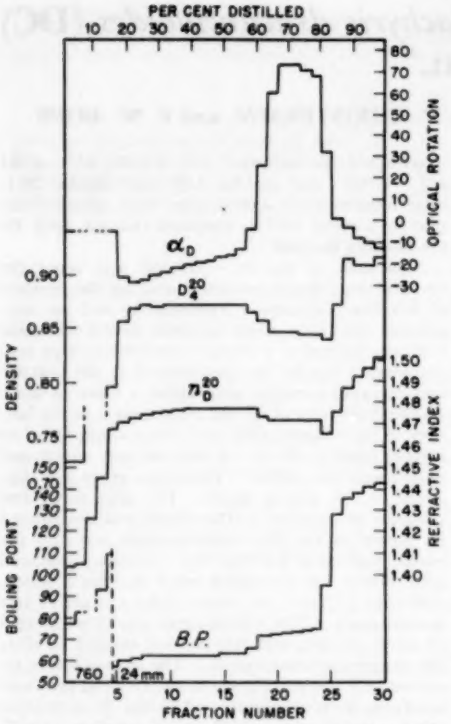

Fig. 1.—Physical properties of the terpene fractions of the oil of *Amphiachyris dracunculoides*.

A summary of the physical properties and the R_f values of the three combined fractions are given in Table I.

TABLE I.—PHYSICAL PROPERTIES AND R_f VALUES OF THE MAJOR COMBINED HYDROCARBON FRACTIONS

Sample	Boiling Point, °C.	Refractive Index, n_D^{20}	Optical Rotation, $[\alpha]_D$	Density, d_4^{20}	R_f
1	165–167	1.4759	−26°02′	0.869	0.72
2	175–177	1.4724	+75°24′	0.843	0.35
3	248–250	1.4965	−08°32′	0.912	0.82

Identification of α-Pinine.—The forerun of sample 1, consisting of fractions 4, 5, and 6 of the hydrocarbon fractionation, was subjected to the action of nitrosyl chloride according to the method of Ehestädt (5). A nitrosochloride was obtained, melting at 102–103° after recrystallization from chloroform-methanol, undepressed by an authentic sample of α-pinene nitrosochloride. The nitrolbenzylamine was prepared from the nitrosochloride by the action of benzylamine (6). After recrystallization from alcohol the melting point of the nitrol was 120–121° undepressed by an authentic sample of α-pinene nitrolbenzylamine.

Identification of *l*-β-Pinene.—Sample 1, consisting of fractions 7–15, did not form a nitrosochloride with the usual reactions. Four grams of sample 1 were oxidized with neutral potassium permanganate according to the method of Délépine (7). The yield of crude acids was 2.6 Gm. Five cubic centimeters of 10% sodium hydroxide was added to the acids with the formation of a white solid, purified by recrystallization from water. The purified sodium salt (150 mg.) was acidified and the free acid recrystallized from benzene-hexane. Nopinic acid, melting point 127.5–128.5°, was obtained.

An infrared spectrogram of sample 1, obtained by means of a Baird Associates automatic recording infrared spectrophotometer, corresponded to that of β-pinene.

Anal.—Calcd. for $C_{10}H_{16}$: C, 88.16; H, 11.84. Found: C, 88.36; H, 11.47.

Identification of *d*-Limonene.—A solution of 1 Gm. of sample 2 in 1 part amyl alcohol and 2 parts anhydrous ether was treated with bromine at 0°. A solid bromide separated out only after long standing in a refrigerator at −15°. The substance was recrystallized three times from methanol to yield a crystalline solid, melting point 103–104° undepressed by an authentic sample of limonene tetrabromide.

The nitrosochloride was formed from sample 2 using amyl nitrite and hydrochloric acid. A solid was obtained melting at 105° after three recrystallizations from chloroform-methanol, undepressed by an authentic sample of limonene nitrosochloride.

An infrared spectrogram of sample 2 corresponded to that of limonene.

Anal.—Calcd. for $C_{10}H_{16}$: C, 88.16: H, 11.84. Found: C, 88.36; H, 11.97.

Identification of *l*-Cadinene.—The physical properties of sample 3 indicated the presence of a sesquiterpene. The molecular refraction was 65.52, calculated for cadinene 66.14. From these facts a bicyclic sesquiterpene containing two double bonds was indicated.

A 2-Gm. portion of sample 3 was dehydrogenated by means of 0.94 Gm. of sulfur according to the method of Ruzika and Meyer (8). The reaction mixture was heated six hours during which time the temperature was gradually raised from 200° to 265°. The reaction mixture was distilled twice at 15 mm. pressure to yield a light yellow oil. The oil was added to 10 cc. of a saturated alcoholic solution of picric acid and the solution warmed on a steam bath. Upon cooling 780 mg. of a picrate was obtained, melting at 114–115°. A sample of 400 mg. of the picrate was split on an alumina column and the freed hydrocarbon was eluted with benzene. Upon removal of the solvent a pale yellow oil resulted n_D^{20} 1.5850, b. p. 289°. Literature values for cadalene are n_D^{20} 1.5851, b. p. 291–292° (8).

The formation of cadalene upon dehydrogenation does not establish the sesquiterpene as cadinene, for other bicyclic sesquiterpenes are known which yield cadalene on similar treatment. One gram of sample 3 was placed in 1 cc. of anhydrous ether and the solution cooled in an ice-salt bath. Dry hydrogen chloride was bubbled into the solution for thirty minutes. The flask was stoppered and placed in a refrigerator at −15°. After three days the flask was removed from the refrigerator and the ether allowed to evaporate. The resulting mash was

filtered to yield a solid in the form of fine white needles. After three recrystallizations from absolute alcohol, 60 mg. of fine white needles were obtained melting at 117.5–118.5° undepressed by an authentic sample of cadinene dihydrochloride.

An infrared spectrogram taken with a Baird Associates Model B infrared spectrophotometer, using sodium chloride standard liquid cell 0.10 mm. in thickness, showed characteristic adsorption bands (see Table II).

TABLE II.—CHARACTERISTIC ADSORPTION BANDS

Cm.$^{-1}$	Microns	Assignment
2,800	3.6	$-CH_2-$ and $-CH_3$
1,635	6.1	$C=C$
1,450	6.9	$-CH_2-$ and $-CH_3$
1,370	7.3	$-CH_3$
885	11.3	$C=CH-$
820	12.1	$C=CH-$
782	12.8	$C=C$

Anal.—Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84. Found: C, 88.07; H, 12.01.

Identification of *l*-Borneol.—The oxygenated fraction was distilled at 27 mm. of pressure and four fractions were collected. A solid appeared in the first three fractions, and after storage at −15° for three days, the fractions were filtered to yield 2.2 Gm. of solid. After two recrystallizations from petroleum ether a crystalline solid was obtained in the form of hexagonal plates, melting at 204° (sealed tube), $[\alpha]_D^{25}$ −39°0′ (c = 2 abs. alc.).

An infrared spectrogram of the solid substance was identical with that of borneol.

The *p*-nitrobenzoate was prepared, and after recrystallization from alcohol melted at 135° undepressed by an authentic sample of bornyl *p*-nitrobenzoate. The 3,5-dinitrobenzoate was also prepared and melted at 156–157° undepressed by an authentic sample.

SUMMARY

The flowering tops of the herb *Amphiachyris dracunculoides* (DC) Nutt. were steam distilled to yield 1.6 per cent of an essential oil with the following properties: d_D^{20} 0.9135, n_D^{20} 1.4784, α_D^{25} −15°36′, acid number 1.06, ester number 26.1, ester number after acetylization 64.8, alcohol content as borneol 10.6 per cent, carbonyl compounds 19 per cent by the bisulfite method. The oil was separated chromatographically on silicic acid into hydrocarbon and oxygenated fractions. The oil is composed of 74 per cent hydrocarbons and 26 per cent oxygenated compounds. The hydrocarbon fraction consists of the terpenes α-pinene 4.5 per cent, *l*-β-pinene 53 per cent, *d*-limonene 24 per cent, and a sesquiterpene *l*-cadinene 18 per cent. *l*-Borneol has been isolated from the oxygenated fraction.

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INTERNATIONAL CONGRESS OF THE HISTORY OF PHARMACY

The International Society of the History of Pharmacy will hold its 1954 general meeting within the framework of the INTERNATIONAL CONGRESS OF THE HISTORY OF PHARMACY in Rome, Italy, September 8–12, 1954. This will be followed by the *International Congress of the History of Medicine*, beginning September 13. The program will include a visit to the Nobile College of Chemistry and Pharmacy, which is observing this year its 525th anniversary.

Members who wish to present papers are requested to submit them to: Herr Apotheker C. Wartenberg, Ploener Strasse 184, Eutin-Neudorf (West Germany), not later than two months prior to the Congress. The papers must be on original subjects and of general interest to the history of pharmacy. They may be presented in German, French, English, Italian, or Spanish, but an abstract in a second language is required. Delivery of a paper must not exceed twenty minutes.

Displacement Analysis of Terpene Hydrocarbons*

I. Preliminary Studies

By A. C. GLASSER,† L. E. HARRIS, B. V. CHRISTENSEN, and F. W. BOPE

Displacement analysis on silica gel has been investigated as a method of separating terpene hydrocarbons. The use of adsorption isotherms in predicting the nature of displacement diagrams has been substantiated. An application of displacement analysis to a mixture of terpene hydrocarbons isolated from a natural source is presented.

DURING the analytical examination of essential oils, limitations of the conventional methods are often encountered. Some of these are the initial volumes required and the lack of sharp separations while working with small quantities utilizing distillation techniques. Chromatographic methods are generally not limited by such considerations of quantity, and are useful in the separation of small quantities of materials. The chromatographic method of displacement analysis as developed by Tiselius (1) has been used successfully for the analysis of such substances as sugars (1, 4), amino acids and peptides (2), fatty acids (3), and phenols (5). Fundamentally displacement analysis is an example of the development of a chromatogram in which a developer, or developer mixture is used to displace more weakly adsorbed substances from the adsorbent, and these are in turn made to migrate from the column in an orderly manner with little or no tailing-off.

Recently Fink, Lewis, and Weiss have described conditions under which aromatic and aliphatic hydrocarbons were separated on silica gel (6). The separation of a mixture of hydrocarbons by means of selective adsorption depends upon the fact that the tendency for hydrocarbons to be adsorbed on silica gel decreases in the order: aromatics > olefins > saturates; also, the capacity of silica gel for adsorbing olefins, in the same range of molecular weights, increases in the order: straight chain < branched chain < cyclic olefin. With this information as a basis, the possibility that the varying degrees of unsaturation and the different structures of the terpene hydrocarbons would lend themselves to separation by displacement analysis was investigated with the intent of finding a method adaptable to the analysis of hydrocarbon mixtures as are found in essential oils.

* Received August 21, 1953, from The Ohio State University, College of Pharmacy, Columbus, Ohio.
Presented to the Scientific Section, A. PH. A., Salt Lake City meeting, August, 1953.
Based in part upon a dissertation submitted by A. C. Glasser to the Graduate School of The Ohio State University in partial fulfillment for the degree of Doctor of Philosophy.
† Present address: The University of Kentucky, College of Pharmacy, Louisville, Ky.

EXPERIMENTAL

The terpenes used in this investigation were purified by fractional distillation over sodium and were stored in amber glass under nitrogen until used. Adsorption isotherms were determined in the usual manner at a temperature of 3°. The column used for displacement was surrounded by a jacket for the circulation of alcohol from a large volume reservoir held at 3° ± 0.5°. The reason for the low temperature was twofold: it is well known that adsorption is greater at low temperatures, and silica gel has a reported isomerization effect upon olefins which is reduced or eliminated at low temperatures (6, 7). An isomerization or catalytic effect of the silicates upon terpenes has also been reported by other workers (8–10). The silica gel used was Davison's No. 950-08-08-226, 60–200 mesh. The column used was Pyrex glass, 1 × 40 cm. with a 250-cc. bulb attached to the top. A capillary stopcock was fused flush to the bottom of the tube in order to minimize the mixing of the eluate after it leaves the column. Refractive index measurements were made by means of an Abbe refractometer. The silica gel columns were packed as slurries in *n*-hexane.

Since ethyl acetate is strongly adsorbed, this substance was first investigated as a displacer. Adsorption isotherms were determined for ethyl acetate, limonene, and α-pinene in *n*-hexane, and are presented in Fig. 1. According to the theory of displacement analysis as developed by Tiselius (1), and presented by Claesson (11), the adsorption coefficient[1] which is fixed by the concentration of the displacer chosen, establishes the steady state necessary for the separation of the zones. A line of constant adsorption coefficient is represented by a line drawn from the point on the isotherm of the displacer, which corresponds to the concentration used, back to the origin. This is represented by the dotted line in Fig. 1. The adsorption coefficients of the substances will be the same at the point on their isotherms at which the line to the origin cuts the isotherm, and thus the steady state will be established determining the order in which the zones will migrate from the column. While the displacement continues, the components adjust themselves to the fixed values of the steady state as determined by the concentration of the developer chosen, and the zones move from the column. In the event an isotherm is of such a slope that it is not cut by the line of constant coefficients, this substance will move ahead of the other zones as a free peak, and is not influenced by the displacer.

[1] The adsorption coefficient is represented as the ratio of amount adsorbed to the concentration (cc.$_x$/Gm.)/C_x.

As is seen in Fig. 1, the choice of a displacer as 20% ethyl acetate in *n*-hexane presents just such a condition in that the line of constant coefficients cuts the isotherm of limonene but not that of α-pinene. Thus if 20% ethyl acetate were used as a displacer for α-pinene and limonene, the α-pinene should emerge as a free peak, and such is seen to be the case in the following separation.

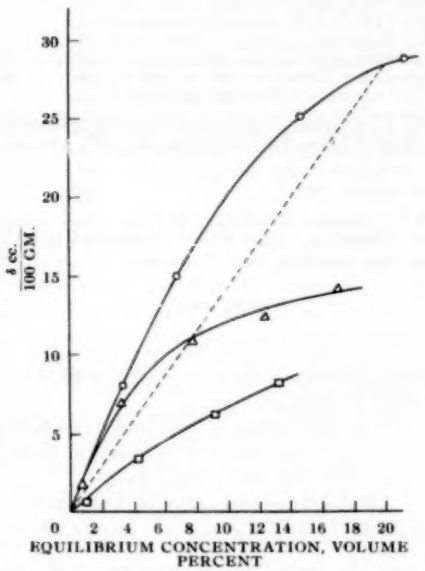

Fig. 1.—Adsorption on silica gel of α-pinene limonene, and ethyl acetate from *n*-hexane. Δ, Limonene; □, α-pinene; ○, ethyl acetate.

Ten grams of silica gel was packed as a slurry in *n*-hexane into the column previously described. A sample of 2 cc. of a mixture of 15% α-pinene, n_D^{20} 1.4660 and limonene, n_D^{20} 1.4724 in *n*-hexane, n_D^{20} 1.3750 was added to the column. The displacer, 20% ethyl acetate, n_D^{20} 1.3719 in *n*-hexane, was added after the last traces of the sample were washed onto the column with *n*-hexane. The rate of flow was adjusted to 0.5 cc. per minute, and the refractive indices were determined at 0.5-cc. intervals. Figure 2 is a plot of the refractive indices *vs.* volume of the eluate. The depression of the refractive index just before the appearance of the α-pinene may be due to a displacement effect on the solvent. It is readily seen that ethyl acetate is too strongly adsorbed to allow a reasonable concentration of this substance to cut both isotherms in Fig. 1. If a steady state is to be established using ethyl acetate as a displacer the concentration of the displacer would have to be quite high in order to enable the line of constant adsorption coefficient to cut the isotherm of α-pinene. A better approach is to utilize a less strongly adsorbed substance as a displacer which would allow the isotherm of α-pinene to be cut at a lower concentration due to the decreased adsorption rather than an increase in concentration. The

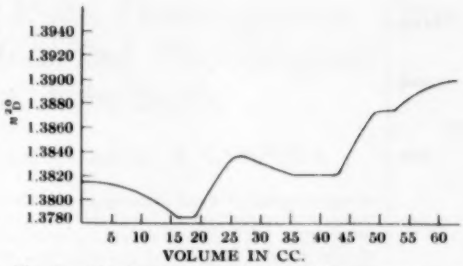

Fig. 2.—Displacement analysis of limonene and α-pinene with ethyl acetate.

aromatic hydrocarbon toluene was utilized for this purpose.

The adsorption isotherm of toluene from *n*-hexane was determined as before, and a composite diagram of the isotherms of toluene, α-pinene, and limonene from *n*-hexane is presented in Fig. 3.

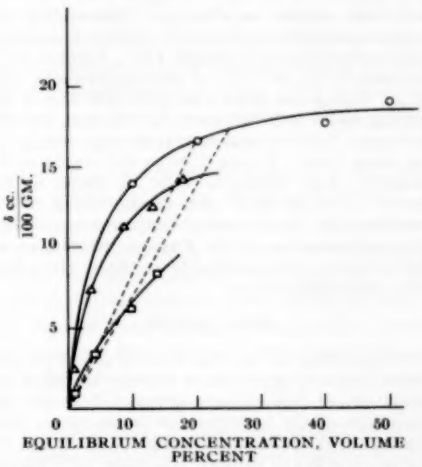

Fig. 3.—Adsorption on silica gel of α-pinene, limonene, and toluene from *n*-hexane. Δ, Limonene; □, α-pinene; ○, toluene.

The line of constant adsorption coefficient from the equilibrium concentration of 25% toluene cuts both isotherms, and thus should resolve a mixture of the terpene hydrocarbons into individual zones in the diagram. A sample of 2 cc. of 10% limonene and 15% α-pinene in *n*-hexane was analyzed on 10 Gm. of silica gel using 25% toluene in *n*-hexane as the displacer. Figure 4 is the diagram of the refractive index *vs.* the volume of the eluate.

It is seen in Fig. 4 that α-pinene no longer runs ahead as a free peak as in Fig. 2, but now is incorporated into the system at a concentration dictated by the point at which the line of constant coefficient cuts its isotherm. This fact is as would be expected because it is seen in Fig. 3 that the line of constant

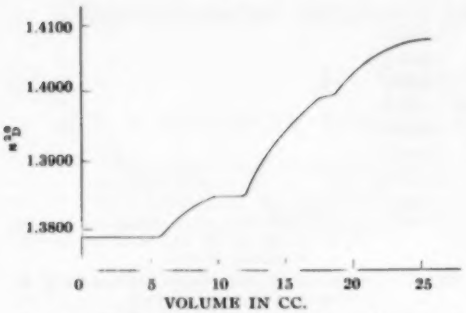

Fig. 4.—Displacement analysis of α-pinene and limonene with toluene.

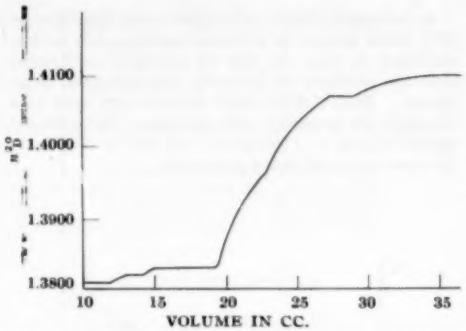

Fig. 5.—Displacement analysis of the terpenes of the oil of *Amphiachyris dracunculoides* with toluene.

coefficient cuts the isotherm of α-pinene. This is in agreement with the theory of displacement analysis as developed by Tiselius (1).

In order to test this method on a more complex mixture of terpene hydrocarbons, 0.5 cc. of the terpene mixture of the oil of *Amphiachyris dracunculoides* was analyzed on silica gel. This mixture has been shown by the authors (12) to have the approximate composition of α-pinene 4.5%, β-pinene 53%, limonene 24%, and 18% of the sesquiterpene cadinene. Ten grams ofsilic a gel was used with a displacing agent of 25% toluene in *n*-hexane, and the procedure followed was essentially the same as in the other trials. Figure 5 shows the results of the analysis. Four breaks or steps are shown on the curve: a small initial step corresponding to the small amount of α-pinene present, a second, larger step corresponding to the β-pinene present, an inflection point corresponding to limonene, and a final step indicating cadinene.

DISCUSSION

It was shown in the experimental procedure presented that changing from a strongly adsorbed displacer to one less strongly adsorbed decreases the separation of the hydrocarbons in agreement with the theory of displacement analysis. However, attention is called to the fact that, while 25% toluene in *n*-hexane allows limonene to appear as a zone in the analysis of a simple mixture, it does not give a definite separation of the terpene in more complex mixtures.

Hagdahl (3) has shown the effect of reduced solubility in the increase in adsorption of fatty acids displaced by the next higher homolog. An incorporation of this effect along with the use of a displacer of intermediate adsorption between ethyl acetate and toluene may allow a more distinct definition of the zone corresponding to limonene.

SUMMARY

1. Adsorption isotherms are given for α-pinene, limonene, toluene, and ethyl acetate in *n*-hexane on silica gel.

2. The application of isotherms in predicting the nature of displacement diagrams of terpene hydrocarbons has been substantiated.

3. Well-defined zones have been obtained in a synthetic binary mixture of terpene hydrocarbons.

4. An application of displacement analysis to a mixture of terpene hydrocarbons isolated from natural sources is presented.

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The Relation Between *in Vitro* Disintegration Time of Sugar-Coated Tablets and Physiological Availability of Riboflavin*

By D. G. CHAPMAN, R. CRISAFIO, and J. A. CAMPBELL

Many *in vitro* disintegration procedures have been suggested, but no attempt appears to have been made to assess their physiological validity in a quantitative manner. In the present study the U. S. P. XIV disintegration test was modified for application to sugar-coated tablets. Simulated gastric and alkaline juices and a form of rubbing action with a rubber disk were found to be essential. The validity of the modified procedure was evaluated by a comparison with measurements of the physiological availability of riboflavin in the tablets as determined by a human bioassay technique. Results showed that tablets which did not disintegrate *in vitro* in sixty minutes were not completely available to the human subject as judged by urinary excretion of riboflavin. An eight-hour urinary excretion test was found, in most cases, to be as reliable as the twenty-four hour test. Satisfactory bioassay results were obtained without the use of special diets.

THE CLINICAL EFFECTIVENESS of tablets and capsules depends on at least two factors. The active principle must be present in the expected or labeled amount and it must be available to the patient. Both the pharmaceutical industry and regulatory bodies appear to have made more progress in developing methods for the determination of the ingredients of tablets, than for assessing their physiological availability. It is most desirable that disintegration tests should reflect the availability of a drug to the patient, but neither the *British Pharmacopoeia* (1) nor the *U. S. Pharmacopeia XIV* (2) have tests which have been evaluated on this basis. However, the U. S. P. XIV states (2) that a coating may be applied to official tablets and capsules, provided that it disintegrates in the alimentary tract and is composed of harmless ingredients.

The importance of physiological availability has been recognized by the War Food Administration in the United States (Oser, *et al.* 3). They have required that the vitamins in products purchased by them, not only be present in sufficient total quantities, but also be in a form completely available to the human when the product is swallowed whole. Oser and associates (3, 4) studied extensively the physiological availability of water-soluble vitamins in tablets and developed a human bioassay technique for the direct determination of availability. The method is based on the urinary excretion following a test dose of the vitamin. More recently, Sobel and Rosenberg (5) measured physiological availability of vitamin A by changes in the serum level.

As pointed out by Kline (6), urinary excretion tests require rather close control of the diets of the test subjects. For this reason they are cumbersome to use as routine procedures. They are also costly and time-consuming. The need for the simplification of these tests or the development of *in vitro* procedures which have been correlated with them is obvious.

Many *in vitro* and *in vivo* tests have been proposed to determine the disintegration time of tablets. A comprehensive review of pharmacopoeial methods with reference to compressed tablets has been compiled by Smith (7). He found that many pharmacopeias described standard disintegration times for compressed tablets varying from five minutes in the *Pharmacopoeia of Holland* to thirty minutes in that of Russia. The U. S. P. provides individual limits which vary from thirty minutes for tablets such as acetylsalicylic acid and atropine sulfate, to four hours for thiamine and ascorbic acid tablets. In the case of coated tablets, the *British Pharmacopoeia* (1953) allows a period of sixty minutes for certain tablets while the U. S. P. XIV mentions availability but does not fix a time limit for disintegration. According to Smith, the *French Codex* and the 1948 supplement of the *Swiss Pharmacopoeia* allow a two-hour disintegration time. Hoehn (8) reviewed the more recent methods for determining the disintegration times of both uncoated and coated tablets *in vitro*. Stephenson and Smith (9) suggested that pharmacopeial standards for disintegration times of sugar-coated tablets should exceed by at least thirty minutes the normal disintegration time for uncoated tablets, but should never be longer than two hours.

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Thompson and Lee (10) reviewed *in vivo* and *in vitro* techniques for determining the site and time of disintegration of enteric-coated tablets. Several workers including Lozinski and Diver (11) Andersen and Junager (12), and Maney and Kuever (13) traced the course of enteric tablets in the human by fluoroscopic and X-ray procedures, the latter correlating their results with an *in vitro* test.

Some investigators attempted to simulate in their *in vitro* techniques conditions which exist *in vivo*. Abbott and Allport (14) used both simulated gastric and alkaline juices as well as slight agitation by a current of air in an attempt to duplicate more closely certain *in vivo* conditions. Hoyle (15) found no advantage in using an artificial gastric fluid in place of water as the disintegrating medium for uncoated tablets, but showed that temperature and speed of movement influenced disintegration time. Filleborn (16) pointed out that simple *in vitro* disintegration tests are of value only if shown to agree with *in vivo* disintegration times. As a result he developed an "artificial stomach" in which he attempted to duplicate gastric juice, *p*H, hydrostatic pressure, and peristalsis as found in the human stomach. Evanson and DeKay (17) introduced a revolving drum into their apparatus which they claimed provided a rolling wave-like action on the tablet similar to that of stomach contractions. This method was found to give a decided advantage in reproducibility of results over the Gershberg and Stoll method (18) which was later modified and adopted by the U. S. P. XIV. In the latter, little attempt was made to reproduce physiological conditions. The agitation is gentle and neither force nor pressure is applied directly to the tablets which are submerged continuously in distilled water. For coated tablets, Howard (19) showed that it was the varnish coat and not the subsequent layers which had most influence on the disintegration of tablets in water.

The limitations of all *in vitro* and *in vivo* disintegration tests and of serial roentgenograms have been pointed out by Oser and his associates (3) who indicated that urinary excretion is the only true measure of physiological availability of the water-soluble vitamins. There appears to be practically no information available on the actual physiological validity, as judged by excretion studies, of any of the disintegration procedures suggested. The importance of the physiological approach cannot be overestimated in the development of *in vitro* procedures. Quantitative excretion data would seem to furnish a much sounder basis for these tests than has hitherto been possible.

Preliminary data obtained in this laboratory suggested that there was a definite advantage in the use of simulated physiological juices rather than water as disintegrating media for sugar-coated tablets. The need for some form of light rubbing action on the tablet also became apparent from this work. The U. S. P. XIV apparatus for the disintegration of compressed tablets, which is simple and widely accepted, was used as the basis for the modified test.

It is the purpose of this paper, therefore, to describe a modified procedure for the disintegration of coated tablets and for the estimation of physiological availability, and to report the results of a study of the relation between them.

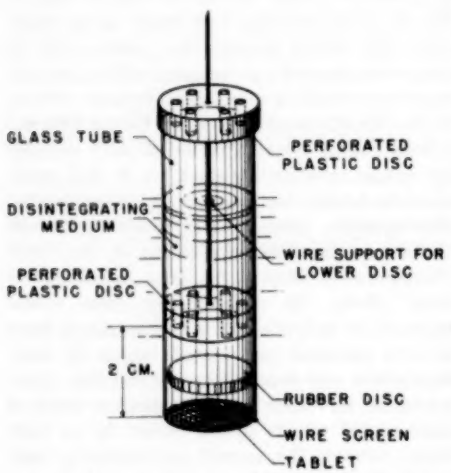

Fig. 1.—Modifications of tube in U. S. P. XIV disintegration test for tablets.

METHODS

Disintegration Test.—*Apparatus.*—The disintegration apparatus used was essentially that of the *United States Pharmacopeia XIV* (2) except that a 3-L. beaker replaced the 1-L. beaker as the disintegration chamber, the tablets were subjected to light rubbing action by rubber disks, and perforated plastic disks were inserted in the tubes to standardize the distance traveled by the tablets and rubber disks. The two latter modifications are shown in detail in Fig. 1. The rubbing action on the tablet was supplied by a disk cut from the large end of a No. 2 rubber stopper. These disks were polished on an emery wheel to a weight of approximately 1.35 Gm. so that they moved freely up and down in the tube. The plastic disk was set at a distance of 2 cm. above the wire screen.

Media.—Simulated gastric and alkaline juices were used to replace water as the disintegrating medium and were prepared as recommended by Ab-

bott and Allport (14). The composition of these juices is shown in Table I. The disintegrating medium was held at 37° by means of a knife-edge heater and a thermostat.

TABLE I.—COMPOSITION OF GASTRIC AND ALKALINE JUICES

Simulated Gastric Juice (*p*H 1.6)	
Sodium chloride	1.4 Gm.
Potassium chloride	0.5 Gm.
Calcium chloride	0.12 Gm.
Pepsin B. P.	3.20 Gm.
1 *N* Hydrochloric acid	190 ml.
Distilled water to make	1,000 ml.
Simulated Alkaline Juice (*p*H 8.0)	
Pancreatin B. P.	2.8
Sodium bicarbonate	15.0
Distilled water to make	1,000

Procedure.—One tablet was placed in each of the six tubes and the rubber disks inserted so that they rested horizontally on the tablets. The plastic disks were placed in position as shown in Fig. 1. The level of the juices was adjusted so that the top of the basket did not go below the surface of the liquid at any time. The basket-rack assembly was immersed in the gastric juice at 37° and the motor was started. If the tablets did not disintegrate in thirty minutes, they were removed from the gastric juice and placed in alkaline juice until all the tablets had disintegrated. The disintegration time for a product was taken as the mean disintegration time of the six tablets. In spite of the larger volume of liquid which was used to reduce the cloudiness, it was occasionally found necessary, with some tablets, to stop the disintegration apparatus and lift the basket out of the medium to observe the state of disintegration. To reduce this cloudiness it was also found helpful to filter the pancreatin solution before adding the sodium bicarbonate and diluting to 1,000 ml. If, as happened occasionally, a tablet stuck between the rubber disk and the tube it was separated manually by means of a long thin spatula.

Physiological Availability.—The method used to determine the physiological availability of riboflavin was essentially that of Melnick, Hochberg, and Oser (4) who showed that there was a linear relationship between the dose of riboflavin and the amount excreted in the urine.

Subjects.—Ten normal males employed in the Food and Drug Laboratories volunteered as subjects. They were shown to be receiving nutritionally adequate diets as indicated by the recovery of the test dose of riboflavin. While on test, they were permitted to continue to consume their regular meals but were cautioned to eat meals which were similar in nature from day to day, and to refrain from eating foods such as liver, which are known to be high in riboflavin. In this respect the technique was different from that of Oser and his associates (4) and Kline (6) who supplied the same diet to all subjects on test.

Assay Periods.—The riboflavin standard or sample tablet was taken at about 8 a. m. immediately after breakfast and the urine was collected for the next twenty-four hours in two portions, the first eight-hour excretion and the second sixteen-hour excretion. Collections were made in brown bottles containing 10 ml. of 3.5 *N* sulfuric acid. A blank was obtained for each standard and each unknown on the preceding day, as follows: *Monday*—The bladder was emptied just before breakfast and the urine collected for the following twenty-four hours in two lots. The riboflavin found in this sample served as the basal excretion for the standard taken the following day. *Tuesday*—Riboflavin, 5 mg., contained in the standard compressed tablet (three minute disintegration time) was swallowed whole immediately after breakfast. The urine was again collected for the next twenty-four hours in two lots. *Wednesday*—No collections were made. *Thursday*—Urine was collected as before and served as the basal excretion for the unknown. *Friday*—An unknown tablet or tablets containing approximately 5 mg. of riboflavin was taken immediately after breakfast and the urine again collected as before.

Where the riboflavin excretion from the standard was not determined each week, the most recent figure was used for comparison with the unknown sample. No difference was noted between the availability calculated from the standard excretion of the same week and that from the mean of all excretion values for the standard.

Determination of Riboflavin.—Riboflavin in the tablets and in the urine was determined by the U. S. P. fluorometric procedure (2). All calculations of availability were based on duplicate determinations of riboflavin in the tablets.

Availability of Riboflavin.—In Table II riboflavin excretion figures are shown to illustrate how the availability, in per cent, was calculated for the twenty-four-hour period. In the same way, by using the eight-hour excretion values for both sample and standard, the availability on the eight-hour basis was determined.

TABLE II.—METHOD OF CALCULATION OF PER CENT AVAILABILITY OF RIBOFLAVIN

Excretion of Riboflavin in 24 Hours

Control Period

Monday, Basal Excretion, mg.	Tuesday, After Dosage of 5 mg. Standard, mg.	Riboflavin from Standard, mg.	Standard Excreted, %
0.88	3.46	2.58	52

Test Period

Thursday, Basal excretion, mg.	Friday, After Dosage of 5 mg. Unknown, mg.	Riboflavin from Unknown, mg.	Unknown Excreted, %
0.72	1.96	1.24	25

$$\text{Per cent availability} = \frac{25}{52} \times 100 = 48\%$$

RESULTS

Disintegration Test.—One of the first questions that arose in this work was whether water or simulated gastric and alkaline juices should be used as disintegration media. Table III presents a comparison of disintegration times of a series of coated tablets determined in the simulated juices and in water. No rubbing action was used in these tests. It may be noted that tablets which have similar dis-

TABLE III.—COMPARISON OF DISINTEGRATION TIME OF TABLETS IN WATER AND IN GASTRIC AND ALKALINE JUICES

Tablet	Time in Minutes	
	Juices[a]	Water
A	30	35
B	45	50
C	45	75
D	60	60
E	65	85
F	75	180[b]
G	90	180
H	90	180[b]
I	165	240[b]

[a] Thirty minutes in gastric juice, balance of time in alkaline juice.
[b] Did not disintegrate.

integration times in the juices may have widely different disintegration times in water alone. Some tablets are so resistant to disintegration in water that it might be questioned if this disintegration time is not affected out of all proportion by the presence of a coating which is insoluble in water alone. In these cases it would seem that such figures are more or less meaningless. Furthermore, since a tablet, in passing through the alimentary tract, is subjected to both acid and alkaline treatment it seemed logical to use these media as the basis for the test. Experience with these tests also indicated that sharper end points of disintegration were essential and suggested the need of some form of rubbing action.

The advantage of using rubber disks to supply rubbing action is shown in Table IV. The disintegration time of six tablets of each of five brands was determined both with and without disks on three separate days. In each case an analysis of variance was carried out on the results obtained. Table IV shows the mean time of disintegration for each brand as well as the average variation within the groups of six tablets as measured by the variance and by the coefficient of variation. The latter is probably the more appropriate measure of variation since the mean disintegration time varied considerably between brands and with or without rubber disks. Judged in this way there was markedly less variation with rubber disks than without them. There was more variation within some brands of tablets than in others. It was noticed that with rubbing action, sharp end points were obtained for all tablets tested. Without rubbing action, end points were more variable and some were unduly prolonged. This was caused by some tablets remaining as soft gummy masses of varying sizes which were too large to pass through the screen. Thus the difference in disintegration time with and without rubbing action varied with the type of tablet.

In order to study possible sources of variation in the recommended procedure three tablets were disintegrated on each of four days and records were kept of the position of each tablet in the basket so that variation in this factor could also be studied. The results of an analysis of variance of the data are shown in Table V. Mean disintegration times for the three types of tablets were 35, 47, and 136 minutes. The test appeared remarkably stable since there was no significant variation between positions or days or in any of the interactions.

Physiological Availability Test.—Twenty-five brands of coated tablets containing riboflavin and representing the production of 15 manufacturers were examined in the present study. The mean disintegration time (*in vitro*) was determined on six tablets from each sample. The physiological availability of the riboflavin in each of the samples was determined after ingestion by each of the ten subjects, or by as many as were available for test at a given time.

In order to determine their suitability for this study each subject was repeatedly given 5 mg. of riboflavin in an uncoated tablet which was known to disintegrate within three minutes and the per cent of the dose excreted was calculated. The average values for each individual for both the eight and twenty-four hour periods are shown in Table VI. The results indicated that all of the subjects were receiving adequate amounts of riboflavin and therefore would be suitable to participate in the project. The mean excretion value for the 10 sub-

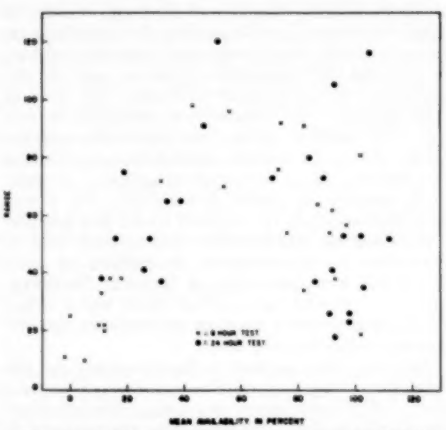

Fig. 2.—Relation between the mean availability of riboflavin in tablets and the range of individual values.

TABLE IV.—EFFECT OF RUBBER DISKS ON MEAN DISINTEGRATION TIME AND PRECISION OF TEST

Tablet	With Rubber Disks			Without Rubber Disks		
	Mean Disint. Time, Min.	Error Variance	Coeff. of Variation, %	Mean Disint. Time, Min.	Error Variance	Coeff. of Variation, %
J	36.0	6.0	6.8	56.1	227.8	26.9
K	38.5	11.6	8.8	55.3	73.3	15.4
L	66.9	107.5	15.4	128.6	758.9	21.5
M	75.2	69.7	11.1	159.4	464.4	13.5
N	88.8	55.2	8.3	152.0	341.7	12.1

TABLE V.—ANALYSIS OF VARIANCE OF DISINTEGRATION TIMES OF THREE TABLETS OBTAINED ON FOUR DAYS

Sources of Variation	D. F.	Mean Squares
Total	71	
Tablets	2	16,604
Positions	5	9.4
Days	3	31.0
Tablets × Positions	10	39.5
Tablets × Days	6	7.1
Position × Days	15	65.7
Tablet × Positions × Days	30	35.1

jects was 39 ± 4% of the standard in the eight hour period and 47 ± 5% in the twenty-four hour period. These results showed that the riboflavin excreted in the first eight hours amounted to 83% of that excreted in twenty-four hours from a readily available tablet. The variation of the eight-hour figures was essentially the same as that of the twenty-four hour values.

Variation between individual subjects in their urinary riboflavin excretion was found to be a much more important factor with some samples than with others. Data obtained on the standard was relatively uniform while on some unknown tablets there was as much as 100% difference in availability between two individuals. The distribution of this variation, expressed as the difference between the maximum and minimum value for each tablet, is shown graphically in Fig. 2. The widest range between individuals obviously occurred in the middle area where one subject utilized a tablet satisfactorily while another subject did not. The eight-hour data show the same trend as the twenty-four hour figures.

TABLE VI.—RIBOFLAVIN EXCRETED IN THE EIGHT AND TWENTY-FOUR HOUR PERIODS AFTER THE INGESTION OF 5 MG. OF STANDARD[a]

Subject	Basal Excretion, mg.	Excretion After Test Dose, mg.	Riboflavin from Test Dose, mg.	Dose Excreted, %
	In 8-hour period			
A	0.415	2.342	1.927	39
B	0.374	2.377	2.003	40
C	0.340	2.388	2.048	41
D	0.382	2.361	1.979	40
E	0.280	2.122	1.842	37
F	0.329	2.029	1.700	34
G	0.300	2.127	1.827	37
H	0.252	2.722	2.470	49
I	0.288	2.073	1.785	36
J	0.553	2.557	2.004	40
	In 24-hour period			
A	0.974	3.359	2.385	48
B	0.703	3.311	2.608	52
C	0.966	3.530	2.564	51
D	1.028	3.101	2.073	41
E	0.850	3.081	2.231	45
F	0.523	2.384	1.861	37
G	0.623	3.035	2.412	48
H	0.350	2.997	2.647	53
I	0.698	2.921	2.223	44
J	1.063	3.481	2.418	48

[a] Figures represent means of 2 to 10 separate determinations.

Relation between Disintegration Time and Physiological Availability.—The detailed data on the *in vitro* disintegration times and the physiological availabilities of the twenty-five tablets examined are shown in Table VII. Mean values for each tablet are plotted in Fig. 3. These results showed that there were few tablets which had a disintegration time greater than sixty minutes and yet were physiologically available. This limit of sixty minutes in disintegration time thus afforded a useful distinction between those tablets which were available and those which were not. Tablets No. 18 and 19 were possible exceptions and were examined further. By repeated analysis of aliquots of the disintegration media it was shown that the riboflavin was leached out of these tablets before they disintegrated. This was further substantiated by the fact that the range between the eight and twenty-four hour values for tablets 16, 18, and 19 was markedly greater than that for tablets which were classed as available, the low eight-hour values presumably being an indication of the slow leaching of riboflavin rather than disintegration of the tablet. There were insufficient tablets of No. 16 to determine if the riboflavin also tended to leach out before disintegrating. In contrast, tablet No. 15 which conformed to the premise showed no appreciable leaching-out of the riboflavin until the tablet disintegrated.

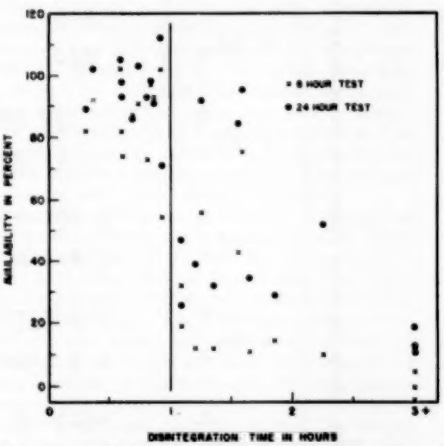

Fig. 3.—Relation between disintegration time of tablets and mean per cent availability of riboflavin.

Data for both eight and twenty-four hour periods are given in Table VII. It appeared that there was good correlation between the methods. With one or two exceptions tablets which have been found to be available by the twenty-four hour test would not be penalized by the eight-hour procedure. Tablets which might be considered to exhibit different availabilities by the two tests were borderline cases and were not fully available to all subjects on the twenty-four hour test.

TABLE VII.—AVAILABILITY OF RIBOFLAVIN FROM SUGAR-COATED TABLETS AS DETERMINED WITH TEN SUBJECTS

Tablet No. →	1		2		3		4		5		6		7		8		9		10		11		12	
Disintegration Time, Min.																								
Rubbing Action →	18		20		35		35		36		40		44		48		50		52		55		56	
No Rubbing Action →	33		20		40		35		52		48		46		54		74		54		58		59	
Excretion Test, Hours →	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24
Subjects	Availability, %																							
A	50	61	47	65	105	147	..	..	..	..	61	70	122	121	..	..	..	..	106	85	96	123	83	107
B	136	134	..	..	..	..	..	..	55	85	83	78	95	100	24	81	77	94	72	86	..	..	81	100
C	55	72	104	96	89	76	..	..	99	111	..	..	..	87	100	98	72	97	..	..	112	102	26	42
D	113	113	..	..	72	103	141	155	81	98	77	107	86	110	70	90	86	117	95	104	101	85	..	..
E	50	72	103	106	63	88	..	39	..	..	125	89	68	103	..	99	129	104	82	78	93	137	66	71
F	..	..	109	114	..	..	..	..	..	..	..	..	..	..	84	97	..	..	..	..	..	..	..	..
G	77	76	94	101	31	42	104	127	..	..	86	91	87	..	..	..	..	..	..	..	..	..	24	52
H	..	..	92	118	32	65	101	122	..	..	..	..	73	86	..	..	..	..	..	..	..	..	13	34
I	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	123	80	..	..	..	..	..	..
J	93	95	94	111	123	128	60	80	..	..	87	80	108	113	87	93	..	..	110	102	105	112	82	94
Means	82	89	92	102	74	93	102	105	82	98	87	86	91	103	73	93	97	98	93	91	102	112	54	71

Tablet No. →	13		14		15		16		17		18		19		20		21		22		23		24		25	
Disintegration Time, Min.																										
Rubbing Action →	65		65		72		75		81		93		95		110		98		186		135		180+		180+	
No Rubbing Action →	82		75		76		79		242		159		112		130		110		245		180+		180+		180+	
Excretion Test, Hours →	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24	8	24
Subjects	Availability, %																									
A	6	16	36	69	5	10	93	94	22	25	..	..	81	106	−5	12	6	−2	8	8	0	0	−4	−3	..	−4
B	42	54	73	98	6	20	13	100	2	25	..	..	82	119	22	62	5	64	−7	45	11	120	3	26	0	30
C	4	13	..	..	..	..	8	68	..	..	9	37	45	85	..	..	2	13	−1	−1	15	21	4	15	10	6
D	13	18	..	..	..	..	..	..	..	..	..	..	56	75	2	14	−2	−1	−5	−2	..	..	−6	−5	5	48
E	24	31	31	33	..	..	4	..	..	..	107	117	88	113	10	11	20	57	−2	−5	11	4	−7	33	5	0
F	..	..	5	26	..	..	88	89	..	..	83	99	99	105	25	42	27	42	14	41	..	..	..	..	..	..
G	..	..	45	51	27	75	..	..	11	20	47	93	78	66	..	..	2	54	6	54	..	..	..	..	..	..
H	..	..	..	..	10	19	100	109	..	..	5	67	..	..	9	10	12	25	−11	−12	..	..	..	..	..	..
I	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	31	41	3	−4	22	94	..	..	..	..
J	..	..	1	7	..	72	89	91	..	57	9	92	..	..	33	45	9	43	−1	63	1	75	..	..	..	..
Means	18	26	32	47	12	39	56	92	12	32	43	84	76	96	14	28	11	34	0	19	10	52	−2	11	5	16

Further data are also shown on the effect of rubbing action of the disks. As noted previously rubber disks reduce disintegration time in some cases. For most of the tablets the range of individual disintegration times for the six tablets is less with rubbing action than without.

DISCUSSION

The subject of disintegration of tablets has been investigated in the past from various approaches. Attempts have been made to simulate *in vivo* conditions in a variety of ways but no *in vitro* method, in itself, can be entirely successful because it is not possible to reproduce completely all physiological conditions. Thus all *in vitro* tests, regardless of how they may attempt to duplicate physiological conditions must be considered to represent a more or less arbitrary or empirical approach to the subject. It is therefore essential that any *in vitro* disintegration test, if it is to be considered valid, must be correlated at some stage with a quantitative measure of physiological availability. Urinary excretion as determined in the human bioassay technique appears to be the most reliable criterion available.

The fact that a relationship was established between the *in vitro* test and physiological availability substantiated the importance of two factors which were incorporated into the disintegration test. Gastric and alkaline juices appeared to be necessary as disintegration media to act on certain constituents of the tablets, especially those present in the coatings. If the coatings resisted the action of a particular medium, the tablet did not break down and the disintegration time was, in some cases, out of all proportion to the availability of the ingredients in the tablet. Slight rubbing action on the tablet was found to be necessary supporting the earlier suggestions of Evanson and DeKay (17). Those tablets which become soft and gummy would be available, yet without rubbing action they have unduly prolonged disintegration times. The test was found to be objective and did not depend on personal interpretation of the disintegration end point.

It is interesting to consider the results of the disintegration test in relation to the physiological availability of riboflavin in various types of tablets. In general, those tablets which disintegrated in sixty minutes or less were available, while those that took longer to disintegrate were not. While availability was considered to be satisfactory if the mean excretion of different individuals was more than 70% of the standard excretion, it was realized that this mean could include individual values representing incomplete utilization. For the exceptions noted, it has been shown that riboflavin had leached out before the tablet had completely disintegrated. This resulted in a relatively high availability in spite of a long disintegration time. Such a tablet would be considered unsatisfactory if judged on the basis of disintegration time. This would seem to be correct, for other constituents might still be unavailable in the absence of complete disintegration. A similar discrepancy between the two tests could exist in a multilayered tablet where the active principle is on the outside of a relatively impervious coat. Obviously the ingredient investigated, in such a case, must be in the inner core of the tablet. On the other hand, a tablet may disintegrate within a reasonable time but as pointed out by Oser, *et al.* (3), the drug may be adsorbed by other constituents of the tablet and not appear in the urine. In such cases, disintegration tests will obviously not reflect physiological availability. Consequently, in comparing these procedures consideration should be given to these factors.

The importance of variation in the response of human subjects should be emphasized. As was shown in Fig. 2, relatively better agreement was obtained between subjects on those tablets which were either readily available or relatively unavailable than on those tablets in the middle part of the availability scale. In Table VII there appeared to be more variation between individual data for unknown tablets than was previously found for the standard preparation. For example, it was noted that subjects B, F, G and J tended to obtain a greater proportion (average 61.7%) of riboflavin from the less readily available tablets, Nos. 13 to 25, than did subjects A, C, D, E and H (average 30.6%). While from a practical standpoint neither of these values represent satisfactory availability, it was of interest to investigate the question further. On studying the rate of riboflavin excretion of the standard preparation it was noted that the former individuals tended to excrete a lower proportion of riboflavin in the first two hours after taking the dose than did the latter group. These data would suggest that physiological differences in individuals may be a factor in their utilization of such preparations. Further reference to Table VII indicates that the variation between individuals is more random in those tablets which are more readily available—that is numbers 1 to 12. The means for the two groups in this case were 93.4 and 95.7%. While these data as a whole serve to illustrate the magnitude of variability of the test, it does not appear to be as important in those tablets which approach either end of the availability scale. Obviously, to obtain reliable results a sufficient number of subjects must be used to allow for differences between individuals.

The advantages of the availability test based on an eight-hour urinary excretion are obvious. While the eight-hour figures are, on the whole, approximately 10% lower than the twenty-four hour values there is essentially no difference in their interpretation of availability. The differences observed between the two values are undoubtedly caused by the fact that the eight-hour data are influenced to a greater extent by the time taken for the tablet to disintegrate in the alimentary tract. In some cases, the eight-hour values agree better with the disintegration tests than do the results of the twenty-four hour tests. While the departure from diets of known weight and composition undoubtedly sacrifices a certain amount of precision the fact that coefficients of variation of determinations on the standard preparation are of the order of 4 to 5% indicates that the test itself is reliable.

No data have been obtained on the general applicability of this test to tablets containing other medicinal ingredients. Oser, *et al.* (3), have shown that several B vitamins and ascorbic acid exhibit similar excretion curves. Since riboflavin is probably the least soluble of those studied, it might be expected that if the riboflavin in a tablet were available other water-soluble vitamins would also be available.

Some of the tablets investigated were enteric coated. These tablets would appear to differ only in the fact that they must withstand some minimum disintegration time in gastric juice as well as meet the disintegration test described. The addition of some minimum criterion for these tablets such as one hour in gastric juice is a possibility, but has not been investigated.

SUMMARY

1. The U. S. P. XIV disintegration test for compressed tablets has been modified for sugar-coated tablets by employing simulated gastric and alkaline juice and rubber disks to provide rubbing action. The validity of this procedure has been assessed by comparison with estimates of the physiological availability of riboflavin in the tablets as determined by the human bioassay technique.

2. It was found that there was a relationship between the disintegration times, as determined by this test, and the physiological availability of the riboflavin in the tablets. Tablets which did not disintegrate in sixty minutes were not completely available to the human subjects.

3. The eight-hour urinary excretion test was found to be much less cumbersome and equally as satisfactory as the twenty-four hour test. Satisfactory bioassay results were obtained without the use of special diets.

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The Efficiency of Dihydroxy Aluminum Aminoacetate in Phosphate Insolubilization*

By ROBERT S. MURPHEY and DAN L. KENDRICK

An *in vitro* comparison of the efficiency of several aluminum type antacids in the insolubilization of phosphate is reported. Included in the study are dihydroxy aluminum aminoacetate, aluminum hydroxide gel and powder, and aluminum carbonate gel. The *in vivo* effectiveness of dihydroxy aluminum aminoacetate in phosphate diversion from urinary tract to intestinal tract is also shown.

THE POSSIBLE VALUE of aluminum compounds in the treatment of individuals with a tendency to formation of renal phosphatic calculi was first suggested by Shorr (1) in 1945. Shorr and Carter (2) reported successful management of this condition in 22 patients by the administration of large doses of aluminum hydroxide or aluminum carbonate gels over periods of as much as seven years. Barrett (3) in a series of 34 patients and Marshall and Green (4) in a series of 37 patients have fully confirmed the findings of Shorr.

Freeman and Freeman (5) first reported in 1941 the successful use of aluminum hydroxide gel for the reduction in concentration of inorganic phosphorus in the serum and urine of a series of children with chronic renal insufficiency. Using 120 cc. of the gel daily they succeeded in correcting hyperphosphatemia and hypocalcemia in the children with uremia. More recently, Page and Page (6) have demonstrated the effectiveness of aluminum hydroxide tablets in the relief of leg cramps in pregnancy through intestinal insolubilization of phosphate, thereby producing a shift in the plasma calcium/phosphate ratio.

* Received August 28, 1953, from the Research Laboratory, A. H. Robins Company, Inc., Richmond 20, Va.

The usual practice in the control of renal phosphatic calculi by employment of colloidal aluminum preparations has been to put the patients on a diet providing a phosphorus intake of 1.0–1.3 Gm. per day. While the normal urinary excretion of phosphorus is about 1.0 Gm. per day it is considered desirable to reduce this to less than 300 mg. per day in phosphatic calculi-susceptible persons. To accomplish this in conjunction with the diet it is necessary to give 160 cc. of aluminum hydroxide gel (3.36 Gm. of aluminum) or 120 cc. of aluminum carbonate gel (3.24 Gm. of aluminum) per day.

Adequate control of the aluminum treatment is facilitated by the use of a simplified method for the determination of inorganic urinary phosphate, which requires only readily available chemicals and equipment. Such a method was described by Taussky and Shorr (7) recently.

It appears that the gels are much more effective than tablets of hydrated alumina in the insolubilization of phosphate in the gastrointestinal tract, which reduces the absorption of phosphate from the intestine and ultimately diminishes excretion of phosphate by the kidneys. For example, experiments by Shorr (1) show the aluminum hydroxide gel to be one and one-half times as effective as the dried powder on the basis of aluminum content. Thus approximately 5.0 Gm. of aluminum in the form of hydrated alumina tablets is required to do the same work as 3.36 Gm. of aluminum in the form of aluminum hydroxide gel with respect to the reduction of the urinary excretion of phosphorus.

Since it is generally known that aluminum hydroxide gels are not uniform in composition and vary with the method of manufacture (1) and since the blandness of the gel becomes monotonous (4) and is distasteful to some persons, it was felt that an examination of the phosphate combining ability of a pleasant-tasting solid preparation of dihydroxy aluminum aminoacetate[1] (DAA) might furnish some significant information concerning the value of this compound as a phosphate immobilizing agent in the treatment of renal phosphatic calculi and in the relief of pain from leg cramps in pregnancy. Several studies (8–13) have shown DAA to be a highly effective antacid, but up to this time no consideration has been given to its possible utility in the control of phosphate absorption. Since the amount of phosphate which can combine with aluminum to form aluminum phosphate is dependent on the amount of aluminum ions present, it follows that the aluminum-type antacid which is most effective as an antacid will also furnish the greatest relative amount of aluminum ions. It has been shown that DAA is a very effective antacid and, therefore, one would expect it to be quite effective also in the immobilization of phosphate. This is now shown to be the case.

[1] Robalate® tablets, A. H. Robins Company, Inc., Richmond, Va.

EXPERIMENTAL

In vitro.—The method used for the reaction of the aluminum compound with artificial gastric juice has been previously described (11). Four powdered DAA tablets were added to a stirred solution of 150 cc. of *p*H 1.5 hydrochloric acid containing 2.0 Gm. per L. of pepsin, N. F., in a thermostatically controlled bath maintained at 98.6° F. At the end of ten minutes, 20 cc. of the acid-antacid mixture was withdrawn and replaced by 20 cc. of fresh acid-pepsin solution. The 20-cc. portions were withdrawn and replaced by fresh 20-cc. portions each ten minutes over a two-hour test period. As each 20-cc. portion was withdrawn it was added to a known excess of monobasic potassium phosphate and the mixture adjusted to *p*H 6.9 by the addition of 10% sodium carbonate solution for maximum precipitation of the aluminum phosphate. The suspension was filtered at once and the concentration of phosphorus remaining in solution was determined spectrophotometrically by the Bodansky method (14) for the determination of serum phosphate. At the end of the two-hour test period the phosphate insolubilizing ability of the remaining 150 cc. of mixture was determined and the total amount of phosphorus bound as aluminum phosphate was calculated. Similar tests were run on equal amounts of aluminum as occurring in powdered hydrated alumina tablets, aluminum hydroxide gel, and aluminum carbonate gel. All determinations were made in duplicate, with close agreement between runs.

In vivo.—While on a normal diet one of the authors (R. S. M.) collected the total twenty-four hour urine each day for seven days and total phosphorus excretion was determined by the Bodansky method (14). After this seven-day control period, with a continuation of the normal diet, the ingestion of DAA Tablets was started, with the tablets being taken at the rate of 16 per day, four after meals and four before retiring. Twenty-four hour urine samples were collected and total inorganic phosphorus determined as before.

RESULTS AND DISCUSSION

Table I shows the results of the comparison between equal amounts of aluminum as occurring in the various antacids considered. Choosing hydrated alumina tablets as the standard for comparison, the relative effectiveness of the various forms of aluminum is shown.

The data shown in Table I indicate that the solid antacid, dihydroxy aluminum aminoacetate, is more effective in the insolubilization of phosphate than is either of the two widely used liquid colloidal alumina preparations and one tablet form of hydrated alumina. Tablets of DAA are more than twice as effective as tablets of hydrated alumina when compared on the basis of equal amounts of aluminum.

TABLE I.—INORGANIC PHOSPHORUS REMOVAL BY EQUIVALENT DOSES OF ANTACIDS

	Aluminum Hydroxide Gel	Hydrated Alumina Tablets	Aluminum-Carbonate Tablets	Dihydroxy Aluminum Aminoacetate Tablets
Aluminum input, mg.	342.0	342.0	342.0	342.0
Phosphorus removal, mg.	139.9	119.0	251.2	327.9
Per cent of standard	117.6	100.0	211.0	275.6

If one calculates the theoretical amount of phosphorus which the aluminum used should remove, then one finds that the DAA tablets actually remove as much as 96% of the calculated amount of phosphorus while tablets of hydrated alumina remove only 43% of the theoretical amount.

While the results of the *in vitro* tests indicate that DAA should be a highly effective form of aluminum for the immobilization of phosphate in the intestinal tract, the proof of its value in this respect is to be found in the lowering of the twenty-four hour urine phosphorus excretion. Table II shows the results of the *in vivo* studies with the subject on an unrestricted diet throughout the control and test periods.

TABLE II.—EFFECT OF DIHYDROXY ALUMINUM AMINOACETATE TABLETS ON URINARY INORGANIC PHOSPHORUS EXCRETION

Day	Normal Diet, Gm./24 Hr.	Normal Diet + 16 DAA Tablets, Gm./24 Hr.
1	1.49	0.565
2	1.85	0.748
3	1.57	0.624
4	1.89	0.614
5	1.51	0.684
6	1.22	0.518
7	1.38	0.608
Av.	1.56	0.609

The calculated aluminum input was 1.37 Gm. per day and the average diversion of 0.951 Gm. of inorganic phosphorus from excretion via the urinary tract to elimination through the intestinal tract represents an efficiency of 60.7%. No effort was made to adjust dosage of DAA or diet to reduce the inorganic phosphorus excretion below the 300 mg. per day level considered desirable in the control of renal phosphatic calculi since the principal objective was to demonstrate the potential usefulness of DAA in those conditions where reduction of urinary excretion of inorganic phosphorus was desirable. The *in vivo* results suggest that less than 16 tablets of DAA (each containing 0.5 Gm. DAA, N. N. R.) would be required to maintain the inorganic urinary phosphorus excretion below the 300 mg./day level if the patients were placed on a diet which limited phosphorus intake to approximately 1.0 Gm./day.

SUMMARY

1. The *in vitro* evaluation of dihydroxy aluminum aminoacetate as an agent for insolubilizing phosphate in the intestinal tract and thereby decreasing urinary phosphorus excretion is reported.

2. On the basis of the aluminum content, dihydroxy aluminum aminoacetate is shown to be more effective than aluminum hydroxide gel, hydrated alumina or aluminum carbonate preparations.

3. Results obtained *in vivo* using one human subject, substantiate the deduction drawn from *in vitro* tests regarding the potential usefulness of dihydroxy aluminum aminoacetate in those conditions where diversion of phosphate from the urinary tract or shift of an abnormal serum calcium/phosphorus ratio is desirable.

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Quantitative Determination of Slightly Soluble Salts by Means of Ion Exchange*

By EINAR BROCHMANN-HANSSEN

An ion exchange method for the quantitative determination of slightly soluble salts has been described. The procedure, which has been applied to a number of chemicals and to two tablet preparations, gives reproducible and reliable results. It has the further advantage of requiring little technical skill on the part of the operator and permits a considerable saving of time.

THE APPLICATION of ion exchange in analytical chemistry is not new. It was suggested as early as 1909 (1) and used occasionally for various analytical purposes during the years to follow (2, 3). However, it was not until Adams and Holmes (4) in 1935 succeeded in preparing the first synthetic ion exchange resins that analytical chemistry began to make extensive use of this new tool.

The introduction during the last few years of ion exchange resins containing various functional groups and varying in physical characteristics, capacity, porosity, degree of ionization, and selectivity has greatly stimulated the interest in this phenomenon, and has resulted in many new contributions to the field of analytical chemistry.

The general procedure for analyzing a soluble salt by means of ion exchange is to pass a solution of the salt through a column containing a cationic exchange resin in the hydrogen form. The acid formed by the exchange of cations is then washed through the column and determined by titration or in some other manner. An alternative method, used mainly for salts of organic bases, makes use of a column containing an anionic exchange resin. The anions of the organic salt will be exchanged for hydroxyl ions, resulting in the liberation of the free organic base which can be washed through the column and determined quantitatively.

These methods are not suitable for salts of low solubility. During an investigation of possible improvements in the analysis of calcium mandelate, we observed that this compound rapidly underwent ion exchange when shaken with a suspension of a cationic exchange resin of the sulfonic acid type. Upon filtration and washing of the resin, the mandelic acid could be recovered quantitatively. This observation led to the investigation of even less soluble salts. The work revealed that many substances which usually are considered insoluble could be determined quantitatively by this very simple technique.

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After completion of the experimental work reported in this paper, G. H. Osborn, *The Analyst*, **78**, 220(1953), has described the determination of certain insoluble salts by means of the same technique.

EXPERIMENTAL

The ion exchange resin used in these experiments was Dowex® 50 − X_2[1] (50–100 mesh). It was activated with 5 *N* hydrochloric acid in a chromatographic tube, having a capacity of about one-half pound of resin. The excess of acid was removed by washing with distilled water until the filtrate showed no turbidity with silver nitrate test solution. The excess of water was removed by suction, but the resin was not allowed to dry out. It was stored in a closed container.

Analytical Procedure.—The salt to be analyzed was weighed into a 125-cc. Erlenmeyer flask, and a weighed amount of activated ion exchange resin was added. After addition of a measured volume of distilled water, the flask was stoppered with a cork, and the mixture was shaken in a mechanical shaker. When the ion exchange reaction was complete, the mixture was filtered through a small but firmly pressed cotton plug at a rate of about 1 to 3 drops per second. The Erlenmeyer flask as well as the resin in the funnel was washed out with several small portions of hot water, totaling about 100 cc. After addition of the indicator the acid in the filtrate was titrated with 0.1 *N* sodium hydroxide.

The amount of substance to be analyzed and the amount of resin needed, the volume of water, as well as the time of shaking, were dependent upon the ease with which ion exchange took place. Occassionally it was necessary to add the water hot or even to heat during the shaking period in order to get complete reaction in a reasonable time. Heat was applied by means of a reflector type heat lamp.

The compounds and preparations analyzed by this method, the conditions imposed, as well as the results of the analyses are summarized in Table I. Methyl red was used as indicator for the titration of strong acids, i. e., hydrochloric acid, sulfuric acid, and nitric acid, whereas phenolphthalein was used for titration of mandelic acid, citric acid, and salicylic acid. Potentiometric titration was found necessary in the case of phosphoric acid. The blank value was negligible (<0.03 cc.) when the shaking was carried out without application of heat if, at the same time, methyl red was used as indicator for the titration. With phenolphthalein as the indicator, a blank value of 0.05 to 0.10 cc. of 0.1 *N* NaOH

[1] 2% divinylbenzene cross-linkage.

TABLE I.—QUANTITATIVE ANALYSIS OF CERTAIN "INSOLUBLE" CHEMICALS AND TABLETS

Compound or Preparation	Amount per Analysis, Gm.	Dowex® 50 Added, Gm.	Vol. of Water Added, cc.	Temp. of Water Added, ° C.	Temp. during Shaking, ° C.	Time of Shaking, Min.	Result by Ion Exchange	Result by Other Method
Calcium mandelate	0.4–0.6	5	25	25	25	10–15	99.77%	99.04[a]
							99.91%	98.22
							.....	99.51
							99.79%	100.3
Calcium mandelate tablets, 1 Gm.	0.4–0.6	5	25	25	25	10–15	1.024 Gm./tablet	1.011[a]
							1.024 Gm./tablet	1.040
Secondary magnesium phosphate	0.20–0.30	5	25	25	25	10–15	97.57%	97.50[b]
							97.36%	97.23
							97.21%	97.23
							97.52%	.....
Secondary calcium phosphate, $CaHPO_4 \cdot 2H_2O$[f]	0.2–0.3	5	25	25	25	15	110.4%	110.5[c]
							110.5%	110.8
							110.7%	110.6
							111.1%	.....
Secondary calcium phosphate tablets, 1 Gm.	0.3–0.4	5	25	90–100	25	15	0.9883 Gm./tablet	.....
							0.9963 Gm./tablet	.....
Tertiary calcium phosphate	0.2	5	25	25	25	15	86.12%	85.91[c]
		10					86.40%	86.08
							86.38%	86.16
Calcium citrate	0.2–0.4	5	25	25	25	15	100.5%	99.00[d]
							100.6%	99.02
							100.5%	99.00
Calcium sulfate·$2H_2O$	0.3–0.4	10	50	90–100	25	15	99.24%	99.20[d]
							99.40%	99.12
Lead sulfate	0.1	10	50	90–100	25	30	99.04%	.....
							99.20%	.....
	0.25	10	50	90–100	90–100	30	99.18%	.....
Lead chloride	0.2	5	50	90–100	25	15	99.42%	99.30[e]
	0.3–0.4	10	50	90–100			99.42%	99.10
							99.30%	
Barium sulfate	0.10	10	50	90–100	90–100	60	99.61%	
							99.80%	
							99.76%	
Bismuth citrate	0.10	10	50	90–100	90–100	180	99.02%	
							99.26%	
Bismuth subnitrate U. S. P.	0.2–0.3	10	50	90–100	25	60	102.6%	
							103.0%	
Bismuth subsalicylate	0.2–0.3	10	50	90–100	90–100	60	98.54%	97.66[a]
							98.29%	97.92

[a] U. S. P. method for calcium mandelate tablets.
[b] Determination as magnesium pyrophosphate, single precipitation.
[c] Determination as magnesium pyrophosphate, double precipitation.
[d] Precipitation of Ca as oxalate, titration with $KMnO_4$.
[e] Precipitation as $PbSO_4$.
[f] The results indicate that this compound has lost one molecule of water of hydration. If calculated as $CaHPO_4 \cdot H_2O$ the result is 99.7%.

TABLE II.—THE EFFECT OF VARIATION IN EXPERIMENTAL CONDITIONS ON THE ION EXCHANGE OF LEAD SULFATE

Lead Sulfate, Gm.	Dowex® 50, Gm.	Volume of Water Added, ml.	Temperature of Water Added, ° C.	Temperature during Shaking, ° C.	Time of Shaking, Min.	Result, %
0.2	5	25	25	25	15	34.3
0.2	5	25	25	25	30	49.7
0.2	5	25	25	25	60	59.7
0.2	10	25	25	25	30	70.8
0.2	10	25	90–100	25	30	80.2
0.2	10	50	90–100	25	30	85.2
0.1	10	25	90–100	25	30	93.1
0.1	5	50	90–100	25	30	94.8
0.1	10	50	90–100	25	15	95.5
0.1	10	50	90–100	25	30	99.2
0.25	10	50	90–100	90–100	30	99.2

was obtained. If heat was applied during the shaking period, the blank value amounted to 0.10–0.15 cc.

DISCUSSION

In the batch method for ion exchange used in the experiments just described, the rate of reaction as well as the final equilibrium will depend upon the solubility product of the compound analyzed (BA) and the equilibrium constant (k_1) for the exchange, a property of the resin (RH).

$$BA(s) \rightleftarrows B^+ + A^- \qquad (I)$$

Solubility product $= S_{BA} = (B^+)(A^-)$

$$RH + B^+ \rightleftarrows RB + H^+$$

$$\frac{(RB)(H^+)}{(RH)(B^+)} = k_1 \qquad (II)$$

By adding Eq. I and Eq. II we get:

$$RH + BA(s) \rightleftarrows RB + H^+ + A^- \qquad (III)$$

$$K = \frac{(RB)(H^+)(A^-)}{(RH)} = k_1 \cdot S_{BA} \qquad (IV)$$

Equation IV is an exact thermodynamic expression for the chemical equilibrium III, provided the quantities in the parentheses are thermodynamic activities. If for qualitative comparison we ignore the activity coefficients, Eqs. I–IV explain quite well the effects of variation in the experimental conditions as follows:

(*a*) The stronger the acid resulting from the exchange of cations, the lower the exchange potential of the resin.

(*b*) Substances with a low solubility product, like barium sulfate, react more slowly and require a larger excess of resin for complete exchange than substances with a higher solubility product. The rate of Reaction II is proportional to the available concentration of (B^+). This concentration is often increased by the hydrogen ion released in the exchange if the anion forms a weak acid:

$$BA + H^+ \rightarrow B^+ + HA$$

As an example we may compare the rate of reaction of lead sulfate and tertiary calcium phosphate. Even though tertiary calcium phosphate has a much smaller solubility product than lead sulfate (1×10^{-25} and 1.8×10^{-8}, respectively), lead sulfate reacts much more slowly than tertiary calcium phosphate. The reason is that the solubility of lead sulfate is decreased by common ion effect as sulfuric acid is liberated, whereas the solubility of tertiary calcium phosphate is increased in the presence of phosphoric acid due to formation of the more soluble secondary and primary phosphates.

(*c*) Increasing the temperature will cause an increase in the rate of solution as well as in the solubility product of most salts. The result will be an increase in the rate of the exchange reaction.

It seems reasonable to believe that by proper variation in the experimental conditions, it may be possible to analyze by ion exchange just about any "insoluble" salt that is capable of ionizing in solution. Table II shows the effect of variation in experimental conditions on the determination of lead sulfate.

CONCLUSION

The ion exchange method for determination of calcium salts is much less time-consuming than the precipitation of calcium as oxalate followed by titration with permanganate. It is simpler and gives more reproducible results than the ether extraction method official for calcium mandelate tablets.

In the case of the phosphates, the precision of the ion exchange method does not quite measure up to that obtained by double precipitation as magnesium ammonium phosphate, followed by ignition, and weighing as magnesium pyrophosphate. This is due to the difficulties inherent in the titration of phosphoric acid. The first end point is the sharpest (about *p*H 4.5) and has the advantage that interference from carbon dioxide is negligible. However, if the preparation contains impurities of other anions, like chloride or sulfate, the most accurate results are obtained by titrating from the primary to the secondary phosphate in the absence of carbon dioxide as described by Dijksman (5).

For the determination of the bismuth compounds, the volumetric titration of the acid formed in the ion exchange reaction of the basic salts is of rather limited value from a practical point of view because the composition of these salts is not too well known. The method may be useful, however, in routine analyses of samples and preparations from a batch whose bismuth content is known.

The obvious disadvantage of the ion exchange method described in this paper is its lack of selectivity. Only in cases where there is an appreciable difference in solubility (calcium mandelate + calcium stearate in tablets) or where the resulting acids have sufficiently different ionization constants (phosphoric acid + hydrochloric acid) is it possible to determine different salts in a mixture.

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An *in Vitro* Study of Various Commercially Available Bulk-Type Laxatives*

II. Physicochemical Measurements of Their Absorption of Common Food Principles

By JACK N. BONE† and L. WAIT RISING‡

A study has been made to determine if the bulk-type laxatives now commercially available would absorb or entrap common food principles representing salt, protein, carbohydrate, and fat. A procedure for evaluating this possibility has been developed. The results indicate that many of the bulk-type laxatives studied absorb or entrap common food principles or at least retard their diffusion through a semipermeable membrane.

MANY OF THE bulk-type laxatives now on the market are hydrophilic substances which form various types of masses (1). These bulk laxatives are usually taken over an extended period of time and the question arose as to whether or not these bulk laxatives would, in some manner, absorb or entrap food principles. This investigation was undertaken to make an *in vitro* study of such a possibility using sodium chloride, glycine, dextrose, maltose, and sodium oleate as examples of the salt, protein, carbohydrate, and fat food principles.

PROCEDURE

The diffusion of the food principle through cellophane dialyzing tubing[1] was used as a means of separating the food principle from the bulk-laxative mass. One end of the tubing was rolled up several turns over a Plexiglass plate and secured with a rubber band in order to form a bag approximately 1⅛ inches in diameter and 12 inches long. Aqueous solutions of the food principles were prepared and placed inside the dialyzing bags, then the bags were immersed in distilled water baths. Dialysis was carried on at room temperature for a period long enough so that in the control samples, consisting of just aqueous solutions of the food principles, the concentration of the food principles inside the bag and in the dialysis bath would be the same. Only a very small proportion of the fatty food principle diffused through the membrane, therefore, a dialysis time of eighteen hours was used in the study of this food principle.

Various types of dialyzing apparatus and procedures were used. In one the bag was immersed in the bath and dialysis carried on without agitation. In another the bags were pulled up and down in the bath by means of an arm attached to an automobile electric windshield wiper motor. In another the entire dialysis apparatus consisting of the bath and the bag containing the dialysis mixture was agitated by means of an International shaking machine. In still another procedure the bag was enclosed in a dialyzing frame which was then immersed in the bath. This frame was made of two stainless steel plates, 12 inches by 4 inches, welded together so that there was a space ¼ of an inch wide between the two plates. The plates were perforated with ½ inch holes, ¼ inch apart. Thus, when the dialyzing bags were placed inside the frames, they were held in a flattened form with the enclosed liquid only ¼ inch in thickness.

Five grams of each bulk laxative were used for each 150-cc. aliquot of the food principle solution. These were mixed and placed inside the dialyzing bags. Blanks were run on each bulk laxative by substituting distilled water for the food principle solutions. Samples of the baths were taken and the concentrations of the food principles determined as follows.

Sodium chloride.—The assay of sodium chloride in U. S. P. isotonic sodium chloride solution (2) was used.

Glycine.—Formol titration. The assay of aminoacetic acid in aminoacetic acid elixir (3).

Dextrose.—The assay of dextrose in U. S. P. dextrose injection (4) was used.

Maltose.—Fifteen cubic centimeters of 1 *N* NaOH and 50 cc. of 0.1 *N* iodine were added to the sample and allowed to stand for fifteen minutes; 25 cc. of 1 *N* H_2SO_4 were added and the sample titrated with 0.1 *N* sodium thiosulfate with starch as an indicator. Each cc. of 0.1 *N* iodine used up was equivalent to 17.15 mg. of maltose.

Sodium Oleate.—The water insolubility of oleic acid produced a physical incompatibility with the aqueous mixtures. Therefore, the fatty acid was converted to the water-miscible sodium oleate to provide a more compatible mixture. The *p*H of the sodium oleate solution was 9.6 and did not change when the bulk laxatives were added. The *p*H of the dialysis baths varied from 7.3 to 9.2. Samples

* Received August 21, 1953, from The University of Washington College of Pharmacy, Seattle, Wash.
Presented to the Scientific Section, A. Ph. A., Salt Lake City meeting, August, 1953.
Abstracted from a dissertation presented to the Graduate School of the University of Washington by Jack N. Bone in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
† Fellow of the American Foundation for Pharmaceutical Education, 1949–1951. Present address: College of Pharmacy, University of Wyoming.
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[1] Cellulose Dialyzer Tubing No. 4465-A2. Wall thickness 0.0016 in. Pore diam. 24 Å. Arthur H. Thomas Co., Philadelphia, Pa.

from the dialysis bath were placed in 250-cc. separatory funnels. Three drops of methyl orange indicator and a small but definite excess of dilute HCl were added. The solution was extracted with three 25-cc. portions of petroleum ether. The petroleum ether extracts were filtered and placed in a tared evaporating dish and evaporated to dryness with a stream of air. The samples were then dried to a constant weight at 60°.

EXPERIMENTAL

Ten bulk laxatives representing the psyllium, tragacanth, carob, and methylcellulose groups were studied for the possibility of their absorbing or entrapping food principles by the methods outlined above. Those studied are designated in the following list by composition rather than by trade names. The words in parentheses indicate the form in which the bulk laxative was marketed.

1. Psyllium (granules)
2. Psyllium, 50%, dextrose, 50% (fine flakes)
3. Psyllium (large flakes)
4. Tragacanth (granules)
5. Tragacanth, 95%; kaolin, 5% (granules)
6. Tragacanth (granules)
7. Carob (granules)
8. Methylcellulose (tablets)
9. Methylcellulose, 75%, psyllium, 25% (tablets)
10. Methylcellulose, 9% (liquid)

The data collected, representing averages of the various procedures with all samples run in triplicate, are shown in Table I. Attention is called to the control. This represents the dialysis mixture in which no bulk laxative was used. The data given represent the values after the correction blanks have been considered.

TABLE I.—PERCENTAGE OF FOOD PRINCIPLE IN BATH AS COMPARED WITH THE CONTROL[a]

Sample Number	NaCl	Glycine	Dextrose	Maltose	Sodium Oleate
1	95	93	97	84	69
2	96	78	99	85	69
3	97	87	100	86	69
4	100	80	97	83	31
5	99	99	99	97	38
6	93	69	80	70	38
7	99	93	97	90	62
8	94	94	97	83	62
9	98	95	99	84	62
10	99	99	100	87	62

[a] Based upon the control as 100%.

DISCUSSION

Most of the bulk laxatives studied absorbed or entrapped the food principles, or at least retarded their diffusion, through a semipermeable membrane, as indicated by a lower concentration of the food principle in the dialysis bath when the bulk laxatives were present in the mixture rather than when they were absent.

The diffusion of sodium oleate through the semipermeable membrane was so slight that the results obtained are not considered to be accurate. However, the results which were obtained show that in the presence of the bulk laxatives less of the fatty principle was found in the dialysis bath than in the controls. In the presence of the bulk laxatives sodium chloride and dextrose averaged 3% less than the controls, glycine 11% less, and maltose 15% less.

If the sodium oleate results are omitted, the psyllium group averaged 9% less than the controls for all the food principles, the tragacanth group 11% less, the carob group 5% less, and the methylcellulose group 6% less.

SUMMARY

1. A study has been made to determine if the bulk laxatives now commercially available would absorb or entrap food principles as represented by the salt, sodium chloride; the amino acid, glycine; the carbohydrates, dextrose and maltose; and the fatty principle, sodium oleate.

2. Most of those studied, representing the psyllium, tragacanth, carob, and methylcellulose groups, absorbed, or at least retarded the diffusion of the food principles through a semipermeable membrane.

3. A simple *in vitro* procedure was devised to make this study. Further studies are being made to determine the possibility of bulk laxatives absorbing drugs and vitamins.

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An *in Vitro* Study of Various Commercially Available Bulk-Type Laxatives*

III. Physicochemical Measurements of Their Influence Upon the Digestion of a Carbohydrate, a Fat, and a Protein

By JACK N. BONE† and L. WAIT RISING‡

A study has been made to determine if the presence of bulk-type laxatives would influence the digestion of a carbohydrate, a fat, and a protein. Procedures have been developed for evaluating this possibility. The results indicate that many of the bulk-type laxatives studied retard the digestion of a carbohydrate and a protein and accelerate the digestion of a fat.

THE BULK-TYPE LAXATIVES are usually taken over extended periods of time and the directions for taking these products state that they should be taken one to three times a day with liberal quantities of water. The directions vary as to the time at which they should be taken, some stating before meals, some with meals, some after meals, and some at bedtime or upon arising. This study was carried on to determine if the presence of the bulk laxatives would have any influence upon digestion similar to the digestion as carried on in the gastrointestinal tract.

PROCEDURE

Carbohydrate.—Three different methods were tried and modified so they could be used in the presence of the bulk laxatives. The first method was a modification of a quantitative method for the determination of amylolytic activity by Willstätter, Waldschmidt-Leitz, and Hesse as described in *Practical Physiological Chemistry* by Hawk, Oser, and Summerson (1). The method (Method I) was modified by adding 5 Gm. of the bulk laxative in 300 cc. of distilled water to the digestion mixture. The bulk-laxative particles seemed to absorb the iodine and make the end point difficult to determine when the sodium thiosulfate was added. The method was modified by adding excess sodium thiosulfate and backtitrating with 0.1 *N* iodine solution.

The U. S. P. (2) describes an assay for the starch digestive power of pancreatin. This method (Method II) was modified by adding 2 Gm. of the bulk laxative in 25 cc. of distilled water to the digestion mixture and noting the length of digestion time in which no blue or violet color was produced when aliquots of the digestion mixture were added to the iodine solution.

Because the presence of bulk laxative in the final assayed solution interfered with an accurate assay of the sample, a procedure (Method III) was developed in which the digestion products were separated from the bulk-laxative mass by dialysis. Studies were carried out to determine the optimum pancreatin-starch ratio, the optimum digestion time, and the optimum dialysis time. Studies showed that the digestion of starch by pancreatin could be stopped by boiling the digestion mixture for two minutes. A 3% cornstarch solution was boiled for five minutes. Seventy-five grams of this solution was placed in a 400-cc. beaker and to it was added 1 Gm. of pulverized bulk laxative. This was allowed to stand one hour or until the bulk laxative was hydrated. Phosphate buffer, 10 cc. of *p*H 6.8, and 1 cc. of 1 *N* sodium chloride solution was added and the temperature brought to 37°. Five cubic centimeters of freshly prepared 0.05% pancreatin solution was added and the mixture allowed to digest at 37° for thirty minutes. Boiling chips were added and the mixture boiled for two minutes. After cooling, the mixture was transferred to a dialyzing bag enclosed in a frame with the aid of 2 portions of distilled water, 5 cc. each. The frame was immersed in a 3,000-cc. distilled water bath and allowed to stand at room temperature for twenty-four hours. A 500-cc. aliquot of the bath was taken and the maltose concentration determined by an iodine-sodium thiosulfate titration. For each bulk laxative studied the following reaction mixtures were used: (*a*) bulk laxative plus starch plus enzyme; (*b*) bulk laxative plus enzyme plus water (blank); and (*c*) starch plus enzyme (control).

Fat.—Four methods were tried and modified before one was found which would give reproducible results. The first method tried was a modification of a quantitative estimation of lipase by Willstätter, Waldschmidt-Leitz, and Memmen (3). Variable results were obtained which seemed to be due to the differences in the degree of emulsification of the olive oil. A method was developed in which a primary emulsion of olive oil, acacia, and water was used as the substrate but reproducible results were not obtained. It was thought that if a substrate could be found which was a definite compound and easily emulsified that perhaps a better method for the determination of lipolytic activity could be developed. Glyceryl monostearate and propylene glycol monostearate were hydrolyzed with lipase

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and satisfactory results obtained. However, when the bulk laxatives were added to the digestion mixtures the viscosity increased to such an extent that reproducible results could not be obtained. Bauer and Wilson (4) in one of their investigations used bentonite magma as the emulsifying agent for olive oil. A method (Method IV) which gave the most reproducible results consisted of placing the following in a 500-cc. glass stoppered flask: 10 cc. distilled water, 0.2 Gm. pulverized bulk laxative, 3 cc. bentonite magma, 2 cc. of buffer (0.66 cc. 1 *N* ammonium hydroxide and 1.34 cc. of 1 *N* ammonium chloride) and 2.5 Gm. of olive oil. This was mixed well and allowed to stand one hour or more to allow the bulk laxative to hydrate. To this mixture at 37° 300 mg. of pancreatin was added and the mixture agitated for exactly one minute. The flask was then placed in a 37° bath and allowed to remain for one hour. Eighty-five cubic centimeters of alcohol and 20 cc. of ether was added, phenolphthalein indicator was added and the mixture titrated with 0.1 *N* alcoholic potassium hydroxide, the number of cc. of which was taken as an indication of the amount of fatty acids liberated from the olive oil. Controls were run in which the bulk laxatives were left out of the digestion mixtures. Correction blanks were run on each of the reagents used and various combinations of these reagents.

Protein.—Studies were made to determine the optimum conditions for pancreatic digestion of casein and included a peptic predigestion study. Digesting the casein with pepsin followed by digestion with pancreatin produced slightly higher titration values than when the casein was digested alone by pancreatin. The increase, however, was not great enough to include a peptic predigestion in the final procedure.

The procedure (Method V) used was to place 200 cc. of distilled water and 0.5 cc. of 1 *N* sodium hydroxide in a 500-cc. graduated cylinder. To those mixtures requiring casein, 100 cc. of 5% casein solution was added. Five grams of bulk laxative was added to those requiring bulk laxatives and 20 cc. of 1.25% pancreatin solution was added to those requiring pancreatin. The volume was adjusted to 400 cc., the *p*H to 8.20, and the temperature to 37°. For each bulk laxative investigated the following samples were set up: (*a*) casein solution alone (no pancreatin or laxative), (*b*) casein plus pancreatin, (*c*) casein plus pancreatin plus bulk laxative, (*d*) casein plus bulk laxative, and (*e*) pancreatin plus bulk laxative.

The above mixtures were agitated at 37° for six hours then a 100-cc. sample removed from each. Each sample was adjusted to a *p*H of 8.20 with 0.1 *N* sodium hydroxide and 10 cc. of formaldehyde added; the number of cc. of 0.1 *N* sodium hydroxide required to adjust the *p*H to 8.20 again was noted. The amount of 0.1 *N* sodium hydroxide required for this final titration was used as an indication of the amount of amino acids released by the pancreatic digestion of the casein. Correction blanks were run on all reagents used. The presence of the casein solution and bulk laxatives in the assayed samples made the use of phenolphthalein as an indicator unreliable. Therefore, a Beckman *p*H meter was used in the *p*H adjustments and titrations.

Because the presence of the bulk-laxative mass in the final analysis mixture often interfered with an accurate assay of the digestion products, a procedure (Method VI) was developed in which the digestion products were separated from the bulk-laxative mass by dialysis as follows: 50 cc. of a 5% casein solution was placed in a 150-cc. beaker, 0.5 Gm. of bulk laxative was added and the mixture allowed to stand one hour or until the bulk laxative was hydrated. The *p*H of the casein-bulk laxative mixture was adjusted to 8.20, the temperature to 37° and 6.3 cc. of a 1% pancreatin solution was added. The mixture was digested for six hours at 37°, boiling chips were added, and the mixture then boiled for two minutes to stop the digestion. After cooling, the mixture was transferred to a dialyzing bag with the aid of 5 cc. of distilled water and immersed in a 500-cc. distilled water bath for sixteen hours at room temperature. The dialyzing bag was cellophane dialyzing tubing,[1] 3/4 inch diameter and 20 inches long, tied at the bottom to form a long bag. The top of this bag was fastened to a short, wide-stemmed glass funnel. The dialyzing bath was a 500-cc. graduated cylinder. After the dialysis period, a 200-cc. aliquot was taken from the bath, phenolphthalein T. S. was added, and the sample brought to a faint pink color with 0.1 *N* sodium hydroxide; 50 cc. of formaldehyde was added, then the number of cc. of 0.1 *N* sodium hydroxide required to bring the color back to a faint pink noted. Samples and correction blanks were set up as described in the previous procedure.

EXPERIMENTAL

Ten bulk laxatives were compared as to the possibility of their influencing the digestion of a carbohydrate, a fat, and a protein by the methods outlined above. These bulk laxatives represented the psyllium tragacanth, carob, and methylcellulose groups of bulk laxatives now on the market. Those studied are designated in the following list by composition rather than by trade names. The words in parentheses indicate the form in which the bulk laxative was marketed.

1. Psyllium (granules)
2. Psyllium, 50%; dextrose, 50% (fine flakes)
3. Psyllium (large flakes)
4. Tragacanth (granules)
5. Tragacanth, 95%; kaolin, 5% (granules)
6. Tragacanth (granules)
7. Carob (granules)
8. Methylcellulose (tablets)
9. Methylcellulose, 75%; psyllium, 25% (tablets)
10. Methylcellulose, 9% (liquid)

The comparative data collected are shown in Table I. All samples were run in triplicate. In this table, the method numbers refer to those described in the procedure. The controls are those digestion mixtures in which no bulk laxatives were present. The results obtained in the control samples, i. e., the number of cc. of 0.1 *N* iodine required, each of 0.1 *N* alcoholic potassium hydroxide required, of 0.1 *N* sodium hydroxide required, etc.,

[1] Cellulose Dialyzer Tubing No. 4465-A2. Wall thickness 0.0016 inches. Pore diam. 24 Å. Arthur H. Thomas Co., Philadelphia, Pa.

TABLE I.—COMPARISON, IN PERCENTAGE, OF DIGESTION IN THE PRESENCE OF THE BULK LAXATIVES WITH THAT OF THE CONTROLS[a]

Sample Number	Carbohydrate			Fat,	Protein	
	Method I	Method II	Method III	Method IV	Method V	Method VI
1	105	86	90	140	96	83
2	73	75	91	145	97	88
3	47	120	88	152	97	90
4	114	50	81	78	83	93
5	103	55	85	80	87	99
6	4	120	75	123	91	88
7	135	86	89	164	97	81
8	139	[b]	87	164	98	100
9	103	[b]	39	191	[c]	100
10	134	[b]	88	126	93	90

[a] Based on the controls being 100%.
[b] End point could not be determined.
[c] Results too variable, pH kept drifting alkaline in presence of bulk laxative.

were taken as 100% and the results obtained in the presence of the bulk laxative compared with the controls.

DISCUSSION

In Method I or carbohydrate digestion considerable trouble was experienced due to the entrapment of the iodine reagent by the bulk laxatives which were present in the final assayed mixture. In Method II the same trouble was experienced as in Method I. This probably accounts for the wide variation in the results which were obtained with no correlation between the bulk-laxative groups and their influence upon carbohydrate digestion.

In Method III on carbohydrate digestion the digestion products were separated from the bulk-laxative mass by dialysis. Therefore, the bulk laxatives were not present in the final assayed mixtures and the results which were obtained were much more uniform than in Methods I and II. The results of Method III indicated that all the bulk laxatives studied, representing the psyllium, tragacanth, carob, and methylcellulose groups, retarded the pancreatic digestion of starch. Sample No. 9 produced quite an alkaline solution when suspended in distilled water. Perhaps this alkalinity was the reason for such a strong influence upon the digestion of the carbohydrate.

In Method IV on fat digestion the results indicated that 8 out of the 10 bulk laxatives studied apparently accelerated the pancreatic hydrolysis of the olive oil. This may have been caused by the bulk laxatives aiding in emulsification or subdivision of the olive oil and thus permitting more of its surface to be exposed to the action of the enzyme.

The results of Methods V and VI showed that in the presence of most of the bulk laxatives studied there was less pancreatic digestion of casein than when the bulk laxatives were not present in the digestion mixture. There was no correlation between the bulk laxative groups and the extent to which they retarded digestion. The Method VI involving separation of the digestion products from the bulk laxative mass by dialysis gave more reproducible results than Method V.

SUMMARY

1. A study has been made upon the influence of bulk laxatives, representing the psyllium, tragacanth, carob, and methylcellulose groups upon the digestion of a carbohydrate, a fat, and a protein.

2. The results indicated that most of the bulk laxatives studied retarded the digestion of the carbohydrate and protein and accelerated the digestion of the fat.

3. There was no correlation between the bulk laxative groups and their influence upon digestion.

4. Procedures for making these studies have been described. Those involving separation of the digestion products of the carbohydrate and protein from the bulk laxative masses by dialysis gave the most reproducible results.

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Spectrophotometric Determination of Acetylsalicylic and Salicylic Acids*

By RANDALL B. TINKER† and ARTHUR J. McBAY‡

A simple and rapid spectrophotometric method of analysis of acetylsalicylic acid and of acetylsalicylic acid tablets or capsules has been developed which is accurate to at least ±0.2 per cent. This method may be used also for the determination of salicylic acid and is accurate to at least ±0.2 per cent.

ACETYLSALICYLIC ACID has been used for approximately 100 years, yet no rapid and accurate method of analysis has been developed. In this paper a satisfactory method of analysis of acetylsalicylic acid and a small amount of one of its hydrolysis products, salicylic acid, is reported.

From time to time various methods of assay, which have been accepted for routine analytical work, have been published in the literature (1–4).

EXPERIMENTAL

Acetylsalicylic Acid Standard.—Acetylsalicylic acid, U. S. P. XIV, was assayed by the present and two former U. S. P. methods. This standard assayed 101.54% and showed no test for free salicylic acid. Nonaqueous titration to potentiometric end point showed 101.24% purity (5).[1]

Salicylic Acid Standard.—Analytical reagent salicylic acid was used as a standard. Assay of this sample by the official method showed 99.83% salicylic acid.

Effect of Solvents upon Standards.—Acetylsalicylic acid and salicylic acid were found to have higher absorbancies and greater stabilities in chloroform solution than in either aqueous or alcohol solutions. In addition, chloroform solutions of both standards exhibited the greatest difference between maximum and minimum absorbancy values for any given concentration. It was found necessary to distill technical or U. S. P. chloroform before using, due to the presence of absorbing impurities. Chloroform A. R. grade is satisfactory without distillation.

Wave Length of Maximum Absorbancy of Acetylsalicylic and Salicylic Acids.—Spectrograms of chloroform solutions of 200 μg./ml. of acetylsalicylic acid and of 50 μg./ml. of salicylic acid were prepared and were examined over a spectral range from 240 to 350 mμ. The maximum absorbancy for acetylsalicylic acid and for salicylic acid was found to be 278 and 308 mμ, respectively, as shown in Fig. 1.

Conformity to Beer's Law.—Chloroform solutions of acetylsalicylic acid and of salicylic acid follow Beer's law. Absorptivity indices for acetylsalicylic acid and for salicylic acid are shown in Table I.

TABLE I.—ABSORPTIVITY INDICES FOR ACETYLSALICYLIC AND FOR SALICYLIC ACID SOLUTIONS

Concentration, m*M*	278 mμ Absorbancy	278 mμ Abs. Index	308 mμ Absorbancy	308 mμ Abs. Index
Acetylsalicylic Acid				
20.00	...	...	0.261	0.01305
10.00	...	...	0.129	0.01290
5.00	...	...	0.065	0.01300
2.00	2.700	1.350	0.026	0.01300
1.00	1.340	1.340	0.013	0.01300
0.50	0.670	1.340	0.006	0.01300
0.20	0.268	1.340	0.000	...
0.10	0.134	1.340	0.000	...
Salicylic Acid				
2.00	1.325	0.663	...	...
1.00	0.662	0.662	...	...
0.50	0.330	0.660	1.930	3.860
0.20	0.132	0.660	0.772	3.860
0.10	0.065	0.650	0.385	3.850

Development of Equations.—Examination of the spectrograms for both acetylsalicylic acid and for salicylic acid shows that at a wave length of 308 mμ, the absorbancy due to acetylsalicylic acid is nearly negligible while the absorbancy due to salicylic acid is maximal.

In order to develop equations for determining the concentration of acetylsalicylic acid and of salicylic acid as a two-component mixture, the following symbols were used:

C_x = *m*M of acetylsalicylic acid
C_y = *m*M of salicylic acid
A_1 = absorbancy at 278 mμ
A_2 = absorbancy at 308 mμ
a = absorptivity index

When a solution containing two ultraviolet absorbing components, x and y, is spectrophotometrically analyzed for component x and for component y, the following relationships are true if the light path is considered to be unity:

$$C_x = \frac{a_{y_2}A_1 - a_{y_1}A_2}{a_{y_2}a_{x_1} - a_{y_1}a_{x_2}} \qquad (1)$$

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[1] The authors wish to acknowledge the assistance of Dr. W. M. Lauter, Professor of Pharmaceutical Chemistry, University of Florida College of Pharmacy in the nonaqueous assays.

TABLE II.—ANALYSIS OF PREPARED SOLUTIONS

		Millimoles Calculated		Millimoles Found		Percentage of Error	
308 mμ	278 mμ	A. S. A.	S. A.	A. S. A.	S. A.	A. S. A.	S. A.
0.261	0.268	20.00	0.0000	20.00	0.0000	0.00	0.00
0.645	0.267	19.90	0.1000	19.88	0.0999	0.10	0.10
1.030	0.267	19.80	0.2000	19.83	0.1998	0.15	0.10
1.414	0.266	19.70	0.3000	19.70	0.2997	0.00	0.10
1.797	0.265	19.60	0.4000	19.58	0.3993	0.10	0.18

$$C_y = \frac{a_{x1}A_2 - a_{x2}A_1}{a_{y2}a_{x1} - a_{y1}a_{x2}} \qquad (2)$$

Equations for determining the concentration of acetylsalicylic acid and of salicylic acid were derived from the following absorptivity indices of Table I:

$$a_{x1} = 1.340$$
$$a_{x2} = 0.01305$$
$$a_{y1} = 0.660$$
$$a_{y2} = 3.860$$

A dilution factor of 100 was used to determine the absorbancy at 278 mμ. This factor is included in the following working equations:

$$C_x = 74.75A_1 - 0.1278A_2 \qquad (3)$$

$$C_y = 0.2595A_2 - 0.2527A_1 \qquad (4)$$

To test the accuracy and the validity of these equations, solutions of acetylsalicylic acid containing small amounts of salicylic acid were prepared. These solutions were compared spectrophotometrically at 308 mμ against a blank of analytical reagent chloroform and the absorbancy readings were recorded. Absorbancy readings for these solutions were recorded at 278 mμ after a 100 × dilution had been made. The results obtained are shown in Table II.

Examination of the percentage of error columns in Table II shows that over a range of 98–100% purity for acetylsalicylic acid this method has an error of less than 0.20%.

Method of Analysis of Acetylsalicylic Acid.—Weigh accurately approximately 360 mg. (2 m*M*) of acetylsalicylic acid and dissolve in sufficient chloroform to make exactly 100 ml. Adjust the spectrophotometer to a wave length of 308 mμ, blank against chloroform, and record the absorbancy of the solution as A_2. Make a 100 × dilution of this prepared solution and record the absorbancy of this solution when blanked against chloroform at 278 mμ as A_1. Substitute these absorbancy values in equations (5) and (6) and solve.

$$\text{A. S. A. mg.} = 1347A_1 - 2.3A_2 \qquad (5)$$

$$\text{S. A. mg.} = 3.58A_2 - 3.49A_1 \qquad (6)$$

Method of Assay of Acetylsalicylic Acid Tablets or Capsules.—Determine the average weight of 10 tablets. Crush at least 10 tablets in a mortar and mix thoroughly. If capsules are to be assayed, empty the contents of at least 10 capsules into a tared beaker, mix thoroughly, and determine the average weight. Weigh accurately an amount of the powdered material equivalent to approximately 360 mg. (2 m*M*) of acetylsalicylic acid. Transfer to a beaker by means of a fine stream of chloroform, add 50 ml. of chloroform, stir thoroughly, and allow to stand for two minutes or until the undissolved material has settled. Filter through chloroform-wetted Whatman No. 1 filter paper into a 100-ml. volumetric flask. Rinse out the beaker, pass the rinsings through the filter, and finally bring the solution in the flask up to the calibration mark with chloroform. Continue as directed under *Method of Assay of Acetylsalicylic Acid*.

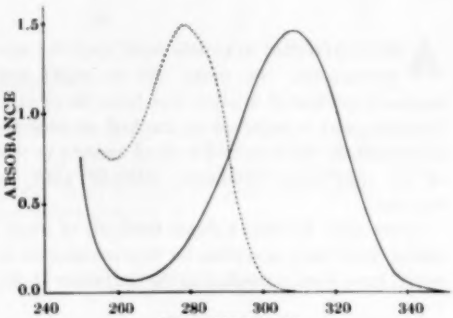

Fig. 1.—Spectrograms of chloroformic solutions of acetylsalicylic acid and of salicylic acid. ..... Acetylsalicylic acid 200 μg. per cc. ——— Salicylic acid, 50 μg. per cc.

Analysis of Market Samples.—Five brands of acetylsalicylic acid tablets were assayed by the above method. The results shown in Table III are the average of three different determinations on each brand of tablets.

TABLE III.—ANALYSIS OF MARKET SAMPLES OF ACETYLSALICYLIC ACID TABLETS, 0.325 GM.

Manufacturer	A. S. A. Found, Gm.	Per Cent of Labeled Amt.	S. A. per Tablet, Mg.
A	0.3254	100.1	0.159
B	0.3019	92.89	0.113
C	0.3244	99.82	0.191
D	0.3248	99.94	0.098
E	Chloroform-soluble coloring interfered		

The use of this method for the analysis of tablets which have chloroform-soluble coloring materials is not advocated at the present time. Attempts have been made to discharge the extracted color from lots of tablets marketed by manufacturers *C* and *E*. The resulting assays were inconsistent within themselves and it was concluded that varying amounts of acetylsalicylic acid had been absorbed on the discharging agent.

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Book Notices

Diabetic Care in Pictures—Simplified Statements with Illustrations Prepared for the Use of the Patient. 2nd ed. by HELEN ROSENTHAL and JOSEPH ROSENTHAL. J. B. Lippincott Co., Philadelphia, 1953. 164 pp. Illustrated. 16 x 23 cm. Price $3.

This is a book that can be recommended to the diabetic patient. It will enable him to understand better the underlying reasons for drugs given to him and regimens prescribed by his physician for the management of the disease. The book is divided into eight chapters, the first of which discusses diabetes and its treatment. In the second chapter normal and diabetic diets are discussed with particular emphasis on the restrictions to be observed by the diabetic and the types of foods he may use. The third and fourth chapters explain insulin and its administration by the patient and signs and symptoms of insulin reactions including the care of the patient with acidosis. In the following chapter the blood and urine tests employed in the clinical laboratory and also by the diabetic are covered. In the last three chapters personal hygiene and special care of certain parts of the body and personal and social factors are presented. The book is profusely illustrated throughout and is provided with an unusually good index. It is definitely written for the patient and in terms that he should be able to understand without difficulty.

British Veterinary Codex, 1953. Published by Direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, 1953. xxiii + 737 pp. 15.5 x 23.5 cm. Price 45*s.* (plus 1*s.* postage).

This Codex is the result of a collaborative effort between the Council of the Pharmaceutical Society of Great Britain, the Royal College of Veterinary Surgeons, and the British Veterinary Association. It has been compiled to supply standards for drugs used in the prevention and treatment of animal diseases and to give authoritative information on their action and uses. Details are provided relative to appropriate doses of drugs which have been found effective in the treatment of various species with special emphasis on horses, cattle, sheep, pigs, dogs, cats, and poultry. Detailed standards for all the important vaccines, sera, and related products used in veterinary practice are also included. Other helpful features of the book are a therapeutic and pharmacological index, a list of trademark names, many useful tables, and over 300 formulas for veterinary dosage forms.

The *British Veterinary Codex* represents a high level type of cooperation between veterinary medicine and pharmacy. The relationship between pharmacists and veterinarians in the United States, while cordial, might be further enhanced if the American Veterinary Medical Association and the American Pharmaceutical Association could agree to emulate the accomplishment of the comparable British societies responsible for the compilation and publication of the *British Veterinary Codex.*

The Pharmaceutical Pocket Book. 16th ed. Published by direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, 1953. 422 pp. 13 x 19.5 cm. Price 18*s.*6*d.*

The usefulness of this book, particularly to British pharmacists, is indicated by the fact that it has undergone fifteen revisions since 1906, culminating in the 16th edition which has recently been published. The purpose of *The Pharmaceutical Pocket Book* is to supply, in summarized form, information relating to nearly all of the aspects of the practice of pharmacy.

The book opens with a comprehensive outline of the activities of the Pharmaceutical Society of Great Britain which are many and varied in character. This information is of special interest to a student contemplating pharmacy as a career. Other sections describe dispensing procedures as practiced daily and include useful information on homeopathy, weight and volume equivalents, tables of official

doses, and a list of abbreviations used in prescription writing.

In addition, methods for calculating the *p*H solutions, the preparation of isotonic solutions, and the emergency treatment of poisoning are also adequately covered. One of the longer sections of *The Pharmaceutical Pocket Book* provides details of various Acts of Parliament and Regulations which affect the practice of pharmacy. Procedures for the examination of urine, blood, cerebrospinal fluid, and examination of gastric contents, are aspects of subjects covered in an unusually competently written chapter on biochemical analysis. Other sections relating to microbiology, antiseptics and disinfectants, infectious diseases, food and diet, with brief descriptions of the vitamins and particulars of the vitamin content of official preparations are also included. The book is replete with much summarized information in tabular form; a useful feature is a dictionary of synonyms containing over 4,000 entries providing ready reference both to official and to obsolete and unusual terms which may be encountered from time to time in the practice of pharmacy. While the book is designed for pharmacists coming under the jurisdiction of the Pharmaceutical Society of Great Britain, it also deserves acceptance as a helpful reference book by pharmacists in other countries.

Elementary Introduction to Molecular Spectra. By BØRGE BAK. North-Holland Publishing Co., Amsterdam (Interscience Publishers, New York), 1954. 125 pp. 14.5 x 21.5 cm. Price $2.90.

Much useful information is presented in the limited scope of 125 pages divided into 5 chapters. The first chapter is devoted to an introductory survey of the main features of spectroscopy approached from a nonmathematical viewpoint. In the following chapter several of the most important equations for an introductory study of theoretical spectroscopy are derived. In the other three chapters applications of these equations within microwave spectra, infrared spectra, and spectra of the visible-ultraviolet region are clearly and succinctly covered. The book stresses throughout the applications and research possibilities of spectroscopy in chemistry and in biology. Information is presented on molecular constitution and thermodynamic properties which is derived from studies of the different types of molecular spectra. The book is well documented with references to the more important textbooks and scientific papers in the field covered by the book. The value of the book is enhanced by an adequate index and a well designed format. The printing and binding are of good quality, and the book should fill a useful purpose as an introduction to molecular spectra.

Quantitative Analysis via Functional Groups. 2nd ed. By SIDNEY SIGGIA. John Wiley and Sons, New York (Chapman and Hall, Ltd., London), 1954. 227 pp. 14.5 x 22 cm. Price $5.00.

Revision of the first edition of this compact treatment of quantitative organic analysis through the determination of functional groups which was published in 1949 has been necessary because this field has developed at an unusually rapid rate during the past five years. The new edition is approximately 40 per cent larger than the first, and includes a new section on some of the techniques and reasoning in developing new methods of functional group analysis and in making changes in known methods in order to analyze samples to which procedures described in the literature cannot be directly applied.

Care has been taken to describe only procedures best suited to general applicability, simplicity, accuracy, and precision and usually requiring only standard analytical equipment. Where special apparatus is required for a few of the procedures it is easy to construct or relatively inexpensive to purchase. The scope of the book can best be appreciated by considering the chapter headings which are: Hydroxyl Groups; Carbonyl Groups; Carboxylic Acids; Salts; Esters; Amides; Imides; and Anhydrides. Alkoxyl Groups; Acetyl Groups; Unsaturation; Active Hydrogen Compounds and Compounds Which React with Grignard Reagent; Acetylenic Hydrogen; Acetal, Ketal-Type Compounds; and Vinyl Alkyl Ethers; Amino Groups; Quaternary Ammonium Salts; Hydrazines; Diazonium Salts; Titanous Chloride Reduction; Mercapto Groups; Dialkyl Sulfides; Alkyl Disulfides; Sulfonic Acids and Salts; Peroxides; Isocyanates and Isothiocyanates; Vinyl Ethers; Oxirane Oxygen (Epoxide Compounds); Water in Organic Groups; Miscellaneous Procedures; Quantitative Separation of Components in a Mixture; Some Physical Chemical Methods Applicable to Quantitative Analysis; Techniques and Reasoning in Developing New Analytical Methods or Modifying Existing Methods; The Role of Quantitative Functional Group Determination in the Identification of Organic Compounds; and Weighing of Volatile or Corrosive Liquids.

Each chapter is documented with pertinent references to the original literature, and the book is provided with an adequate index.

The Indian Pharmaceutical Codex. Vol. I (Indigenous Drugs). By R. MUKERJI. Council of Scientific and Industrial Research, New Delhi, 1953. x + 431 pp. 17 x 25.5 cm. Price *Rs.* 12/−.

This book is designed to serve as a companion volume to the *Indian Pharmacopoeial List*, 1937, [reviewed in THIS JOURNAL **37**, 44(1948)], particularly with reference to the drugs indigenous to India which are described therein. *The Indian Pharmaceutical Codex* bears a resemblance to both the *British Pharmaceutical Codex* and to the *United States Dispensatory*, but differs in many respects from either treatise. The book is arranged in two parts, the first of which consists of general monographs on natural products and drugs of vegetable and animal origin. Part II is a formulary of preparations of these drugs, the aim of which is to promote uniformity in nomenclature and composition. An interesting feature of the monographs on many drugs little known outside of India, is the inclusion of information regarding their chemistry, pharmacologic action, and empirical therapeutic uses. This information should serve to stimulate further research work on many drugs indigenous to India which have been, in many cases, only partially investigated.

Pharmacology. 4th ed., 1953. By J. H. GADDUM. The Oxford University Press, New York, 1954. xviii + 562 pp. Illustrated. 14.5 x 23 cm. Price $8.00.

This textbook is intended for medical students at a stage in their education before general principles become obscured by a mass of practical details. Throughout the textbook facts with an immediate practical application are especially emphasized, but accounts of experimental methods which have led to the introduction of many new therapeutic measures and to the use of potentially dangerous drugs are not ignored. As an example of the practicability of the author's treatment of the subject, the kind of evidence that justifies the trial of new drugs is stressed. In addition to the usual information contained in a textbook of pharmacology, Gaddum's treatment of the subject is unique in many respects and contains much information of interest, not only to medical students, but also to students of pharmacy and related sciences. The book is documented, to a limited extent, by references to articles for supplementary reading, and is provided with a good index. The printing and binding are of good quality, and the book can be recommended as one of more than ordinary interest.

Black's Medical Dictionary. 21st ed. By JOHN D. COMRIE and WILLIAM A. R. THOMPSON. (Adam and Charles Black, London, 1953.) The MacMillan Company, New York, 1953. 1,013 pp. Illustrated. 15.5 x 22.5 cm. Price $7.00.

For almost half a century, this medical dictionary has held a special place in its field due to its skillful balance between the technical and the popular approach on the one hand, and between the characteristics of a dictionary and of an alphabetically indexed encyclopedia on the other hand. The information contained in this new edition is—as in the preceding ones—correct, clear, concise, and up to date. Some 400 illustrations enhance the value of this relatively inexpensive work which should have a wide appeal to physicians, pharmacists, and laymen alike.

Detergency Evaluation and Testing. By JAY C. HARRIS. Interscience Publishers, Inc., New York, 1954. 210 pp. Illustrated. 13 x 18.5 cm. Price $3.75.

This small book is another of the Interscience Manuals designed to provide a brief but adequate description of laboratory procedures in several branches of science and technology. The methods described in this latest member of the series are those which have been found useful in years of experimental work and have been employed in distinguishing between useful agents and those unlikely to be of value as detergents. Minor phases of detergent testing have not been included if they are new and still unproved or have not been sufficiently widely used to warrant their inclusion. The chapter headings indicate the scope of the book and include the following titles: Introduction; Screening Tests; Cotton Washing; Cotton Wash Test Methods; Wool Washing; Washing Procedures for Other Fibers; Hard Surface Cleaning; Radioisotopic Tracer Method; and Miscellaneous Tests.

Poisonous Plants of India. By SIR RAM NATH CHOPRA, RATTAN LALL BADHWAR, and SUDHAMOY GHOSH. Vol. I. (Scientific Monograph No. 17 of the Indian Council of Agricultural Research.) Manager, Government of India Press, Calcutta, 1949. liv + 762 pp. Illustrated. 19.5 x 25.5 cm.

Although this book was published in 1949, a review copy was received by THIS JOURNAL only recently. The object of the book is to furnish a complete outline of the botanical, chemical, pharmacological, and economic aspects of poisonous plants, especially from the point of view of their practical importance. About 700 plants poisonous to man, livestock, insects, and fishes, are covered. The vernacular names, botanical descriptions, distribution, and where known, important chemical constituents and physiological action, the symptoms produced by them, and the modes of treatment and prevention of poisoning are given for each of the 700 plants considered in the book. The poisonous plants are arranged in families according to the Bentham and Hooker systems of classification.

The importance of compiled information regarding poisonous plants is obvious, and this book should serve as a useful reference in the medical, pharmaceutical, and agricultural sciences.

Pharmacognosy Laboratory Manual. By MAYNARD W. QUIMBY, RAYMOND W. VANDERWYK, and WILLIAM E. HASSAN, JR. Burgess Publishing Company, Minneapolis, 1953. 108 pp. 21.5 x 28 cm., spiral bound, paper back.

This manual is "designed to acquaint the student in the pharmacognosy laboratory with drugs and drug products of biological origin, i.e., those produced from plants or animals." The material is presented in three sections: (*a*) Some special techniques comprising 7 pages of instruction and exercises on micromanipulation and microchemical procedures as well as extraction methods for some plant principles. (*b*) Harmful plants, consisting of 12 pages of material on dermato-toxic, poisonous, and allergenic plants, and information on pollen study technique. (*c*) Drugs and economic products of biological origin, containing 85 pages of directions on macroscopic, microscopic, and microchemical means of determining drug identity and purity. Questions are interspersed throughout the exercises to focus the student's attention on his observations and tests. Twenty outline pages to be completed by the student are entitled Prescription Specialties and list the headings: Product Name, Manufacturer, Dosage Forms, and Principal Ingredients.

The material presented represents an inclusive and thorough study of crude drugs and their derivatives. The drugs are arranged according to the taxonomic system of classification rather than the biochemical. Of the recognized subjects of the modern pharmacognosy course the authors include Antibiotics, Allergenic Plants, and Atmospheric Pollen Counts, but they omit information on pollen identification, and insecticidal, rodenticidal, and phytocidal substances. The manual includes typographical and mechanical errors in addition to those listed on the page of "Errata."

Rat Quality A Consideration of Heredity, Diet and Disease. Proceedings of the Symposium held at Columbia University, College of Physcians and Surgeons, New York, N. Y., January 31, 1952. The National Vitamin Foundation, Inc., New York, 1953. 138 pp. 15.5 x 23 cm. Paper bound. Price $2.50.

These proceedings are the result of a meeting held in January, 1952, at the College of Physicians and Surgeons of Columbia University in New York. The book includes the papers presented during the formal program, comments made from the floor, and reports submitted by individuals participating in the symposium. Experiments in the breeding and raising of rats and the relative efficiencies of stock and special diets are rarely published despite their value to other workers. In some of the formal papers included in this book, comparison is made of various commercial rat diets with respect to their nutritional contributions. Perhaps unfortunately, such diets are indicated by code rather than by name to prevent unfair public comparison that would serve no scientific end. This drawback is compensated for by the author's willingness to furnish such information to research workers who desire more precise information on the titles used when specifically requested by letter. The record of the symposium proceedings should serve a useful purpose in clarifying many problems and characteristics of various strains of rats which have heretofore not been well known.

Dictionary of Organic Compounds—The Constitution and Physical and Chemical Properties of the Principal Carbon Compounds and their Derivatives, together with the Relevant Literature References. (In four volumes.) 2nd ed. SIR IAN HEILBRON and H. M. BUNBURY, Editors-in-Chief. Oxford University Press, New York, 1953. Vol. I: Abadole–Cyrosine, 654 pp. Vol. II: D. A.–Hystazarin, 845 pp. Vol. III: Ibogaine-NW-Acid, 838 pp. Vol. IV: Obaculactone–Zymosterol, 694 pp. 21.5 x 27 cm. Price $78 the set.

This *Dictionary of Organic Compounds*, the first edition of which was published between 1934 and 1937, in three volumes, is familiar to many organic chemists in the United States. A new revised edition appeared in 1943 and 1944, and the present new, revised edition in four volumes was completed in July, 1953. In general, the design of the new edition follows that of its predecessors. All of the organic compounds listed are arranged in strictly alphabetical order. Thus any required data may be located readily without the necessity of consulting a supplementary index. In order to use the *Dictionary* with facility, however, it is necessary first to study the arrangement of the text which is clearly explained in the introduction. This is true because so many compounds may be designated in a variety of ways by either trivial or strictly systematic chemical names. The ease of locating required information is enhanced by the generous use of cross references, sometimes as many as three or four. In each short monograph the full structural formula is given for the compound listed. Functional derivatives are usually given under the parent compound unless they are sufficiently important to justify separate inclusion as main references. In each monograph literature references citing the best method of preparation are included.

The order in which data are presented is, in general, the same in all entries. These data include sources, where of interest; physical properties (melting point, boiling point, solubility, density, refractive index, heat of combustion, optical rotation, etc.) and chemical properties. Among the chemical properties the most important include typical reactions and analytical tests. It must be realized, of course, that in many instances only a limited number of chemical and physical properties have been recorded in the chemical literature and are unavailable for listing in the *Dictionary*.

During the course of the revision leading to the publication of the latest edition, each of the previous 15,000 entries was carefully scrutinized and brought up to date. This resulted in the addition of more than 2,500 new principal entries. Many of the changes incorporated include results of research even up to and including the early part of 1953. In the new edition, the type area has been appreciably increased without detracting from the ease of handling.

By the judicious use of the *Dictionary*, the time required in literature searches may be reduced considerably, and it is predicted that the new edition will enjoy an even more popular reception than did its predecessors.

Sergei N. Winogradsky, His Life and Work—The Story of a Great Bacteriologist. By SELMAN A. WAKSMAN. Rutgers University Press, New Brunswick, N. J., 1953. 150 pp. 14.5 x 22 cm. Price $4.

This is a biography of the Russian bacteriologist whose works are particularly associated with studies on the autotrophic bacteria, or those organisms which obtain their energy by oxidation of inorganic elements and compounds. He also contributed to the knowledge of nonsymbiotic nitrogen-fixing bacteria, bacterial oxidation of sulfur, bacteriology of cellulose decomposition, and methods of soil microbiology. References to his 60 published papers are appended.

Glutathione—Significant References from the Pages of Biological Abstracts. Compiled by F. A. VELAY. Biological Abstracts, Inc., Philadelphia, Pa., January, 1954. 19 pp. 21.5 x 28 cm., Price $0.75.

This is the first effort at compiling special bibliography, undertaken by Biological Abstracts and a highly successful one. The topic is timely, the subject arrangement has been done expertly, and the format is perfect. A short historical introduction enhances the value of the list. It is to be hoped that future compilations will maintain the high standards set by the first one.

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Sheraton Plaza Hotel	5.85 up	9.00 up	11.00 up	25.00 up
Somerset Hotel	7.00–10.00	10.00–14.00	12.00–15.00	18.00–40.00
Touraine Hotel	5.50– 8.00	8.00–10.00	8.50–12.00	18.00
Vendome Hotel	4.50– 6.00	7.00–10.00	8.00–11.00	12.00–20.00

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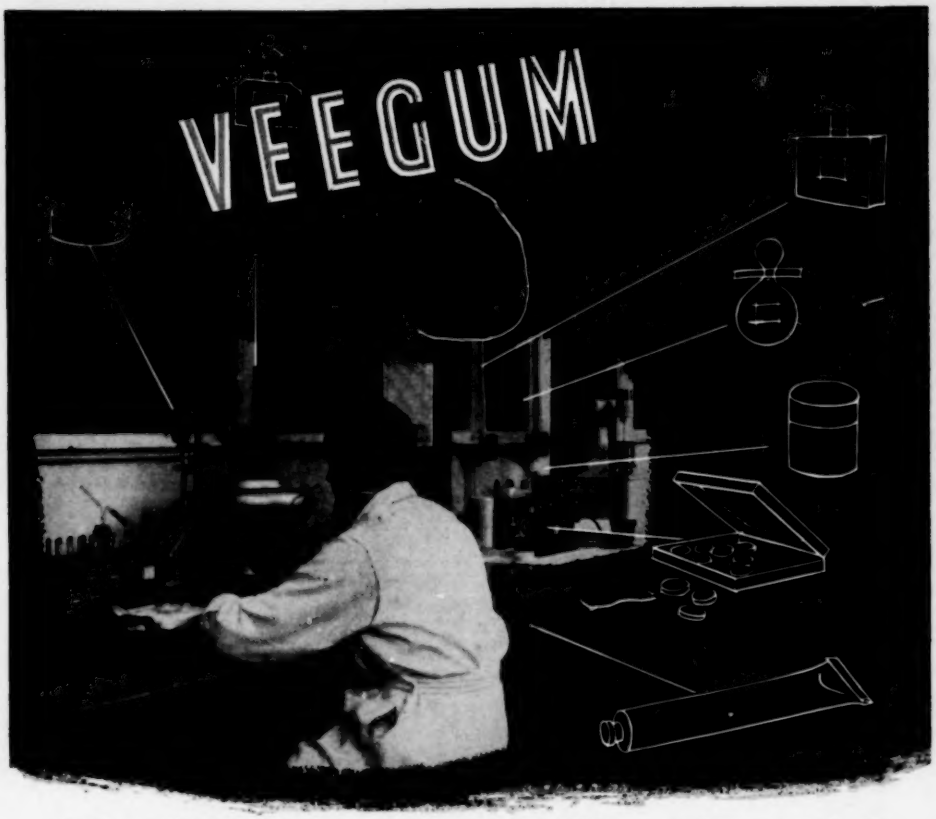

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